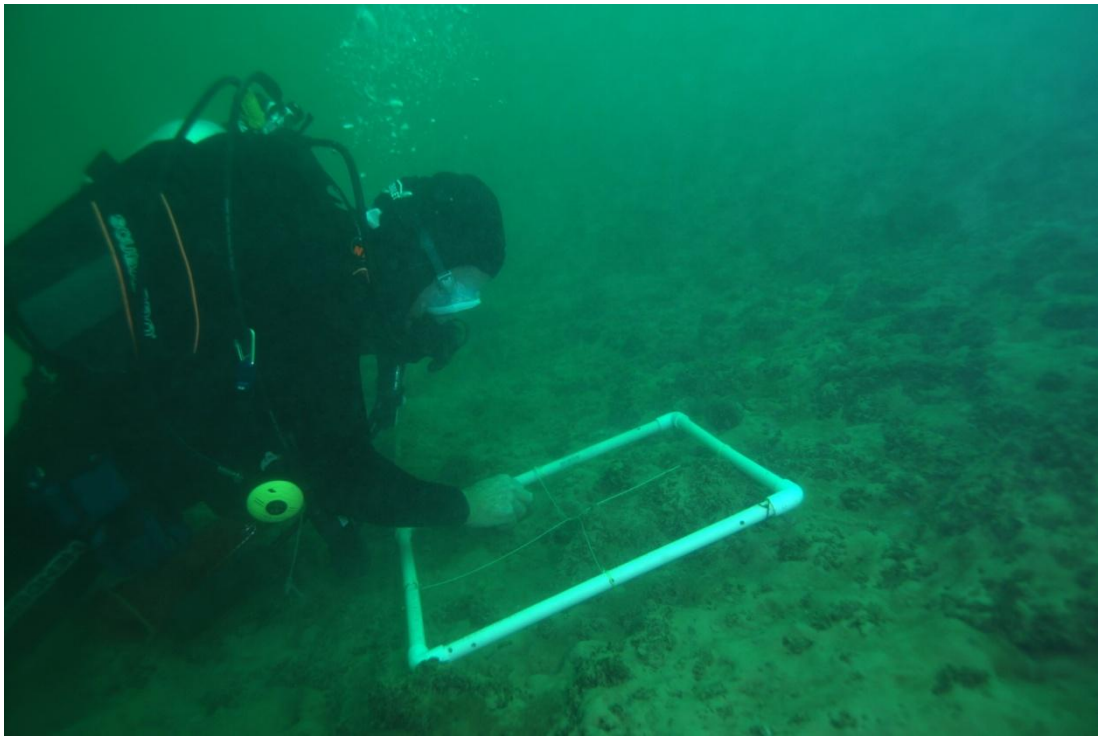
Interagency Monitoring Action Plan (I-MAP):

Quagga Mussels in Lakes Mead and Mohave

Updated 6/01/2011

– Approved Working Document –



Original Submission Date: 9-16-2009 • Revised 6-01-2011

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Members of the I-MAP Quagga Mussel Coordination Team between 2007 and 2009 are gratefully acknowledged for their input into this document. Special thanks are owed to **Jim LaBounty** (Southern Nevada Water Authority), **Peggy Roefer** (Southern Nevada Water Authority), **Todd Tietjen** (Southern Nevada Water Authority), **Chris Holdren** (U.S. Bureau of Reclamation), **Bryan Moore** (National Park Service), **Sudeep Chandra** (University of Nevada, Reno), and **Craig J. Palmer** (University of Nevada, Las Vegas) for constructive discussions and suggestions. Mark Sappington (National Park Service) provided technical assistance. This work was carried out through a Great Basin Cooperative Ecosystem Studies Unit task agreement between the National Park Service and the University of Nevada, Las Vegas. It was supported by Southern Nevada Public Land Management Act (SNPLMA) funding awarded to the National Park Service, Lake Mead National Recreation Area.

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AWWARF	American Water Works Association Research Foundation
AZFGD	Arizona Fish and Game Department
BBAMP	Boulder Basin Adaptive Management Plan
BOD	Biological oxygen demand
Chl-*a*	Chlorophyll *a*
CMT	SCOP Core Management Team
COD	chemical oxygen demand
CWC	Clean Water Coalition
DPBs	disinfection byproducts
DRI	Desert Research Institute
EPA	U.S. Environmental Protection Agency
GPS	global positioning system
Hg	Mercury
HVOD	Hypolimnetic volumetric oxygen depletion
I-MAP	Interagency Action Plan: Quagga Mussels
IOC	Issue of concern
LMNRA	Lake Mead National Recreation Area
MWD	Metropolitan Water District of Southern California
NDOW	Nevada Department of Wildlife
NPS	National Park Service
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenol
PCR	Polymerase chain reaction
ROV	Remotely Operated Vehicle
SCOP	Systems Conveyance and Operations Program
Se	Selenium
SNWA	Southern Nevada Water Authority
THMs	Trihalomethanes
UNLV	University of Nevada, Las Vegas
UNR	University of Nevada, Reno
USBR	U.S. Bureau of Reclamation
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
UV	ultraviolet

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Chapter 1 Executive Summary:
Interagency Objectives and Needs for Monitoring and Management

Following the discovery of quagga mussels in Lake Mead, a variety of agencies, including National Park Service (NPS) Lake Mead National Recreational Area (LMNRA), Bureau of Reclamation (USBR), Southern Nevada Water Authority (SNWA), Nevada Department of Wildlife (NDOW), U.S. Fish and Wildlife (USFWS), Clean Water Coalition (CWC), and U.S. Geological Survey (USGS) have set up monitoring programs to evaluate and gain information to help minimize the impacts or potential impacts of quagga mussels to their facilities and lake ecology. Current monitoring activities and anticipated environmental impacts are depicted in Figures 1 and 2. While the agencies have worked closely and shared monitoring data and findings from the beginning of the infestation, there has been no documented comprehensive monitoring program to describe and record the various quagga mussel-related monitoring needs relative to interagency objectives for Lakes Mead and Mohave. Ad hoc interagency quagga mussel meetings attended by representatives of the above-listed agencies and others served as a springboard for gathering the information with which to establish this Interagency Monitoring Action Plan (I-MAP), which outlines agency objectives related to quagga mussel monitoring and provides approaches to realize these objectives. At the time of this document's publication, the "I-MAP quagga mussel coordination team" included representatives from the following agencies: LMNRA, USBR, SNWA, NDOW, CWC, AZGFD, USFWS, MWD, UNLV, DRI, and UNR (Figure 3). I-MAP team members and their respective agencies provide technical, logistical, and financial support in monitoring quagga mussels and their environmental impacts to Lake Mead. The purpose of the I-MAP team is to coordinate monitoring relevant to the I-MAP.

The goal of this effort is to develop a standardized, long-term, cost-effective, and consistent monitoring plan for quagga mussels in Lake Mead to inform various agencies of the needs and objectives outlined below, and to gain efficiencies from shared operations and information. The plan attempts to build upon current monitoring activities and capabilities, identifies the next steps that can occur within existing capabilities, and, finally, outlines gaps and areas of future need. The first step prescribed by the I-MAP is to identify key needs, questions, and projects related to the objectives associated with (1) infestation of quagga mussels and (2) the basic biology and ecology of quagga mussels. To this end, it is necessary to identify and maintain specific individuals who are willing to review and provide comments (or coordinate the reviews and comments of their respective agencies) in these areas (Figures 4 and 5). It also documents standardized protocols, existing or proposed, for monitoring The I-MAP is a living document and, as such, is expected to be modified over time.

Agency Needs and Objectives

Agencies with direct managerial authorities and responsibilities for Lakes Mead and Mohave include but are not limited to LMNRA, USBR, SNWA, CWC, NDOW, AZGFD, and USFWS. The following section summarizes agency objectives, needs, and/or interests related to rigorous, scientific quagga mussel monitoring for population dynamics and ecosystem impacts to enhance resource management:

NPS Maintaining healthy populations of fish and wildlife; maintaining food-web dynamics; providing high quality, water-based recreation experiences (aesthetics); preventing quagga mussel fouling of boats slipped at marinas; preventing quagga mussel fouling of -- and the resulting increased maintenance of -- facilities such as docks, drinking water intakes, and water treatment plants; developing and adopting best management practices including educational/outreach actions to prevent spread to other drainages and bodies of water; and assessing the potential of control measures.

USBR Preventing quagga mussel fouling of facilities including Hoover and Davis Dams; maintaining healthy populations of native fish in accordance with the goals of the Lower Colorado River Multi-Species Conservation Program; and assessing the potential of control measures.

SNWA Protecting drinking water quality; preventing quagga mussel fouling of facilities including water intakes and treatment plants; and assessing the potential of control measures.

NDOW Maintaining healthy fish (native and sportfish) and wildlife populations; maintaining food-web dynamics; providing high quality recreational boating; developing and adopting best management practices to prevent spread to other drainages and bodies of water; preventing fouling of and resulting maintenance on the fish hatchery within Lake Mead; assessing the potential of control measures; preventing spread to Overton Wildlife Management Area; identifying maintenance and anti-fouling measures if spread to the Overton Wildlife Management Area occurs.

AZGFD Maintaining healthy fish (native and sport fish) and wildlife populations; maintaining food-web dynamics; providing high quality recreational boating; developing and adopting best management practices to prevent spread to other drainages and bodies of water, assessing potential of control measures.

USFWS Maintaining healthy fish (native and sport fish) and wildlife populations; maintaining food-web dynamics; maintaining and preventing fouling of the Willow Beach Fish Hatchery; developing and adopting best management practices including educational/outreach actions to prevent spread to other drainages and bodies of water; and assessing the potential of control measures.

CWC Preventing fouling and need for maintenance of Systems Conveyance and Operations Program (SCOP) facilities, understanding quagga mussel nutrient and limnological interactions to meet objectives of the Boulder Basin Adaptive Management Program (BBAMP).

Major Initiatives Providing Additional Management Influences for Quagga Mussel Monitoring in Lake Mead

There are two significant initiatives that have outlined quagga mussel monitoring needs for Lake Mead. The first is the Boulder Basin Adaptive Management Plan (BBAMP), the initiative sponsored by the permitted wastewater dischargers within Clark County, Nevada to provide an alternative to the Las Vegas Wash for conveyance of treated sewage effluent to Lake Mead while protecting Boulder Basin as drinking water resource, maintaining the health of Lake Mead ecosystem, and keeping the recreational value of the area. The second is the initiative led by SNWA and the Metropolitan Water District of Southern California (MWD), in cooperation with the American Water Works Association Research Foundation (AWWARF) to establish a monitoring, research, and control program for quagga mussels for water-use agencies along the lower Colorado River.

1.2.1. Boulder Basin Adaptive Management Plan (BBAMP) and Quagga Mussels

The CWC established SCOP as a means to improve the manageability of the effluent discharged to the Colorado River system by developing a deep-water outfall effluent pipeline in the Boulder Basin of Lake Mead. To ensure that project purposes of maintaining existing high water quality of Lake Mead are met, the SCOP process developed BBAMP to establish the baseline conditions and management of the operations of the wastewater treatment and the SCOP facilities. It also recognizes the need for identifying and managing non-effluent related stressors in the Lake Mead ecosystem. The BBAMP technical advisory committee has recently outlined items of concern (IOCs) and quagga mussels are related constituents of all these IOCs:

Drinking Water Protection. Through their selective feeding behavior, Quagga mussels have the potential to directly change the species composition of phytoplankton communities and nutrient dynamics by lowering the nitrogen/phosphorus ratio in Lake Mead. The resulting changes may favor the growth of cyanobacteria. If so, water quality will be severely impacted as some cyanobacteria can produce neutrotoxins, hepatotoxins, dermatoxins, cytotoxins, and endotoxins. The distribution of organic and inorganic contaminants (e.g., polychlorinated biphenyls and mercury) and the concentration of total organic carbon will be changed accordingly. Large scale die-offs of mussels can also worsen the water quality. Quagga mussels can clog water intakes and pipes.

Phosphorus in Lake Mead and below Hoover Dam. Through filtration and excretion, quagga mussels can change nutrient cycling in Lake Mead by increasing dissolved phosphorus and decreasing particulate phosphorus in the water column. Therefore, the phosphorus below Hoover Dam could be indirectly affected by the spread of quagga mussels in Lake Mead.

Ecosystems of Las Vegas Wash, Las Vegas Bay and Boulder Basin. Quagga mussels in Las Vegas Bay and Boulder Basin can cause widespread problems and wreak havoc on the Lake Mead ecosystem by, for example, increasing cyanobacteria abundance, altering the nutrient ratio, perturbing the benthic community through fecal pellet production, and disrupting fisheries (see Chapter 2 for more detail).

Recreation Quality with Las Vegas Wash, Las Vegas Bay and Boulder Basin. Quagga mussels damage boats. High mortality rates of quagga mussels on above-water rocks and beach areas result in decomposition, which presents aesthetics and odor problems that impair recreational use of Lake Mead. Sharp shells present a safety hazard to visitors. Large quantities of fecal materials produced by quagga mussels and the associated algae growth in the shallow areas can result in increased bacteria (e.g., *E. coli*) concentrations in nearby beach waters.

Therefore, monitoring and management of quagga mussels in Lake Mead is necessary to meet some information needs within each of the IOCs of the BBAMP.

1.2.2. American Water Works Association Research Foundation (AWWARF) Quagga Mussel Workshop

AWWARF (sponsored by SNWA and MWD) hosted an interagency workshop to discuss strategies for the control and monitoring of quagga mussels to meet agency needs along the lower Colorado River. Workshop attendees outlined the following 14 recommendations for research:

1. Determination of Variability in Quagga Mussel Veligers and Assessments of Chemical Treatment Efficacy
2. Hydraulic Effects on Veliger Mortality in Engineered Systems
3. Quagga Mussel Vulnerability Assessment and Response Management Tool Development
4. Demonstrate Alternative, Non-Chemical, Control Technologies for Quagga Mussels for Deployment at Water Treatment Facilities
5. Molluscicides and Biocides for control of *Dreissenid* mussels in Water Resource Projects
6. Coatings and Materials for Control of *Dreissenid* Mussel Attachment in Water Resource Projects
7. Response of Quagga Mussel Veligers to Limnological Variables
8. Application of Biological Agents to Control Quagga Mussels
9. Applying Knowledge of System Ecology in Control Strategy

10. Quantitative Tools for Management of Mussels in Colorado River System
11. Quantitative Evaluation of Quagga Mussel Outreach and Education Activities
12. Shifts from Planktonic to Benthic Regimes in Response to Quagga Mussel Invasion
13. Early Detection Methodology and Rapid Assessment Protocols for Quagga Mussels
14. Impact of Quagga Mussel Invasion on the Quality of Domestic Water

Monitoring of quagga mussel populations in Lakes Mead and Mohave is necessary to respond to the AWWARF workshop recommendations 1, 3, 4, 7, 9, 9, 10, 12, and 14. Monitoring of related limnological and ecosystem components is necessary to answer 1, 3, 4, 7, 8, 9, 10, 12, and 14.

Three Broad Categories of Agency Objectives for Quagga Mussel Monitoring

The overall interagency objectives for monitoring quagga mussels and the associated ecological responses to their infestation can be delineated within three broad categories.

A. Ecological effects: fish and wildlife
B. Water quality impacts related to drinking water and recreational experience
C. Quagga mussel prevention, control, and infrastructure maintenance

To be most useful, the long-term monitoring program, at a minimum, should include monitoring questions and document information needs for each of these three categories. In order to provide complete information, monitoring is needed for adult quagga mussels, juveniles, and veligers, as well as for additional biota (e.g., benthic organisms, fish, and plankton) that may be impacted. Baseline water-quality and limnological information is necessary to provide a full understanding of impacts.

The following sections outline current monitoring objectives within each of these three focus areas. Identified are immediate needs that the participating agencies feel that they can be met within current capacities and monitoring gaps that will require additional resources.

1.3.1. Understanding and Quantifying Quagga Mussel Effects on Ecology: Fish and Wildlife

Category A relates directly to agency objectives for maintaining healthy populations of fish and wildlife. It also relates to water user and facility questions, such as influences of quagga mussels on meeting BBAMP objectives

and assessing potential control technologies. This is also associated with the potential impacts on bioaccumulation and biomagnifications of organic and inorganic contaminants in the food web. A broad understanding of ecological effects was noted as necessary at the AWWARF workshop.

1.3.2. Water Quality Impacts from Quagga Mussels Related to Drinking Water and Recreation Experience

Category B relates to potential impacts to drinking source water quality, including changes in nutrient loading, changes in taste and odor, and any increase in cyanotoxins. Water quality related to recreation includes impacts to recreational aesthetic qualities such as clarity, but also negative impacts to beaches including odors from decaying mussels, shell hazards, and the presence of algae mats.

1.3.3. Quagga Mussel Prevention and Control / Infrastructure and Maintenance Needs

Category C relates to the prevention of quagga mussel attachment to water facilities, boats, and other structures; stopping spread to other water bodies; and direct control on veligers and adults. This category also includes public education and outreach activities to inform boaters and citizens of the impacts of these invasive organisms and inspire them to engage in behavior that help lake managers to prevent the further spread of mussels to other un-infested waters.

Infrastructure and maintenance needs refers to the severe infestation on dams and docks, water intake and pipeline clogging, as well as maintenance impacts to recreational infrastructure on Lakes Mead and Mohave. This includes information to inform and improve best management practices for boating education and management to prevent spread of quagga mussels from Lake Mead to other water bodies, and best management practices to assist recreational boaters in maintaining vessels free from quagga mussel attachment.

Chapter 2: Potential Ecological and Economic Impacts of Quagga Mussel Invasion into Lake Mead

When quagga mussels were discovered in Lake Mead on January 6, 2007, it extended the U.S. range of this non-native species over 1000 miles west of previously known populations. By the summer of 2007, monitoring data showed that quagga mussels had infested both Lakes Mead and Mohave. Dreissenid mussel (zebra and quagga) invasion of lakes and rivers of North America has already resulted in severe ecological and economical impacts (Nalepa & Schloesser 1993, Connelly et al. 2007). For example, following the 1998 invasion of the Great Lakes by zebra mussels, it is estimated that regional economic damages on the order of $4 billion were incurred in the first 10 years, largely from losses of sport fisheries (Roberts 1990). Before quagga mussels were found in western states, the economic loss due to the invasion of zebra and quagga mussels was already thought to be as high as $1 billion per year in the U.S. (Pimentel et al. 2005). It is estimated that, between 1989 to late 2004, approximately $267 million was spent on preventing dreissenid mussels infestation on electric generation and water treatment facilities in North America (Connelly et al. 2007).

2.1. Potential Ecological Impacts of Quagga Mussels

The health of the Lake Mead ecosystem could be in peril of profound and permanent change due to the presence and spread of the invasive quagga mussel: Quagga mussels are efficient ecosystem engineers that primarily influence the ecosystem by filtering large volumes of water and changing the benthic habitat. The conceptual model below depicts the potential ecological impacts of quagga mussels on water quality and food webs within Lake Mead (Figure 6).

Briefly, quagga mussels can affect any trophic level or limnological parameter in Lake Mead. The potential ecological consequences are explained below.

1. Chlorophyll concentration will decrease and phytoplankton composition in Boulder Basin may change. Before quagga mussel invasion, in Boulder Basin, different groups of algae (i.e., cyanobacteria, green microalgae, diatoms, dinoflagellates, and chrysophytes) are dominant at specific times of the year (LaBounty & Burns 2005). Historically, the Lake Mead open water phytoplankton community is dominated by cyanobacteria in terms of concentration and by diatoms in terms of biovolume. After invasion, the community can shift to a more cyanobacteria-dominated system. Dreissenid mussels selectively choose green microalgae as food, which enhances the ability of cyanobacteria to thrive and reproduce. Perhaps

more importantly, through excretion, dreissenid mussels lower lake N:P ratios in the direction of benefit to cyanobacteria such as *Microcystis*, though the broad spread of most effects on multiple trophic levels mainly radiates from the top-down control of quagga mussels on phytoplankton (both by preying upon them and competing with them), it has been suggested that, in the long term, the change of phytoplankton community caused directly by mussel's selective feeding is less significant than that caused indirectly by mussel's nutrient excretion (Zhang et al. 2008).

2. Other suspended particles, such as silt and detritus, will decrease to some degree due to filtration by quagga mussels. Water will become clearer (i.e. increased Secchi depth) due to the decrease of most suspended particles (e.g., phytoplankton, zooplankton, silt, and detritus).

3. The biomass of zooplankton may decrease: (1) a quagga mussel-caused food shortage will indirectly affect zooplankton growth and reproduction; (2) quagga mussels directly prey upon microzooplankton such as rotifers and copepod nauplii.

4. Dissolved nitrogen (DIN) and dissolved phosphorus (DIP) will increase because of mussel excretion and as an indirect result of decreased utilization by phytoplankton. In turn, the nutrient cycle will be changed: quagga mussels usually excrete less nitrogen and more phosphorus thereby reducing N:P ratios.

5. Aquatic plants, as well as macroalgae such as *Cladophora* in shallow areas, will benefit from the increased water clarity and increased dissolved nutrients.

6. Oxygen will decrease due to its direct consumption by quagga mussels. The loss of some dissolved oxygen can be compensated for by the photosynthesis of increased aquatic plants. However, the consumed oxygen is not replaced entirely as aquatic plants are not abundant in Lake Mead's epilimnion, and few plants exist in the hypolimnion.

7. Organic and inorganic materials (i.e., feces and pseudofeces) produced by quagga mussels will sink to the benthic community.

8. Benthic biomass will increase due to high quagga mussel production. Some species (e.g., Asian clam, *Corbicula fluminea*) may suffer due to direct competition for food resources and habitat. The shells of quagga mussels and increased benthic aquatic plants may provide new habitats for some benthic species.

9. The fishery in Lake Mead may be in peril because striped bass, a top game fish that comprises approximately 70% of the fishery in this lake, may decline if its primary prey fish, the threadfin shad, decreases due to shortages in zooplankton and phytoplankton. Some fish, such as common carp, that can

use quagga mussels or other benthic organisms as food and aquatic plants as habitats, will benefit. The habitat for razorback suckers, an endangered species, may be degraded by the presence of mussel beds in areas previously used for spawning.

10. The concentration of contaminants in the water will decrease because quagga mussels can accumulate in their tissue these organic and inorganic materials from both suspended particles and dissolved phases. Possibly, these accumulated contaminants can be further transferred into higher trophic levels, such as fish and waterfowl, or even human beings who frequently consume fish.

Between 2002-2006 (pre-invasion) and 2007-2008 (post-invasion), no significant change was found in nutrient levels (i.e., ammonia nitrogen, orthophosphate, and total phosphorus), zooplankton abundance, and abundance of threadfin shad. However, the annual concentration of Chlorophyll *a* decreased 49%. Understanding this change is confounded by a concomitant decrease in lake phosphorus levels due to changes in return effluent management practices. TOC in epilimnion decreased and DO in the hypolimnion increased in the post-quagga mussel period, most likely because of the decreasing primary production in the epilimnion. Quagga mussels will change the structure and function of the Lake Mead ecosystem by transferring materials and energy from the pelagic zone to benthic and littoral communities. The precise magnitude and persistence of any changes are unclear at this point. The key factors to determining the degree of consequence are the abundance and distribution of quagga mussels in Lake Mead. At last calculation (in 2007), the average Lake Mead density of quagga mussels was 505 ± 667 mussel/m^2 (Moore et al. 2009), which is still low compared to the almost 10,000 mussel/m^2 in Lake Erie (Patterson et al. 2005). However, it is critical to continue to monitor their abundance and distribution to better anticipate their level of ecological impact in this body of water.

2.2. Potential Economic and Infrastructure Impacts

Dreissena species are biofoulers that can impact not only the ecosystem but also the local economy. *Dreissena* species-related economic impacts resulting from the infestation of power plant water systems, infrastructures, and navigational devices, as well as economic impacts to protecting and maintaining drinking water treatment and recreational assets such as sports fisheries, are tremendous. Following discovery of quagga mussels in Lake Mead, agencies now budget for this nuisance species and are responsible for quagga mussel monitoring, remediation, strategies, and measures taken to prevent future infestation. Some of the following actions have already been employed in, or may be used for, coping with quagga mussels in Lakes Mead and Mohave:

1. Establishment of educational and disinfection programs for recreational boaters to prevent introduction to new waters;

2. Chemical treatment of drinking waters;

3. Construction of new coating materials for water intakes and associated equipment, or new intakes;

4. Physical removal of quagga mussels colonized in water pipes, dam gates, boats, ferries, or other highly infested infrastructures;

5. Setup of monitoring programs to assess the impacts and potential impacts on drinking water, infrastructure, and the ecosystem;

6. Contracts with consulting companies to minimize the impacts;

7. Research projects on the needs to monitor, control, and prevent quagga mussels; and

8. Meetings and workshops to update and share information on quagga mussels.

Although there are no accurate numbers describing how much has been spent on quagga mussel management by multiple agencies, based on the following expenditures in 2008 from several federal agencies (Table 1), it is clear that the impact is severe in terms of direct cost (e.g., control and prevention) and indirect loss (e.g., potential fishery decline). Significant funds will be spent on dealing with this new invader to the western states. For example, USBR spent $ 800,000 for research in 2008 and will double this budget in fiscal year 2009 (Wirkus 2008).

Chapter 3: History and Summary of Findings to Date

In the past two years since the quagga mussel was discovered in Boulder Basin of Lake Mead, progress has been made in monitoring quagga mussel veligers, juveniles, and adults, and in monitoring their potential ecological consequences.

3.1. Findings in Adult Monitoring

1. **Discovery and detection:** On January 6, 2007, the quagga mussel (*Dreissena bugensis*) was found in Boulder Basin of Lake Mead, the largest reservoir by volume in the USA. They first presented in Boulder Basin and later spread to other basins. By the end of 2007, juvenile and adult mussels had been found throughout Lake Mead.

2. **Abundance and distribution:** A whole lake survey in 2007 showed that the average density of quagga mussels in Lake Mead was 505 individuals/m^2. There were more mussels in rocky areas (624 individuals/m^2) than in silty areas (80 individuals/m^2). The deepest area where quagga mussels were found is 108 m (355 ft) and is located in the narrows between Boulder and Virgin Basins.

3. **Size and growth:** Size frequency of mussels collected in 2007 demonstrated that there were always three to four cohorts for each population (Figure 7) with a shell-length range of less than 1 mm to 25 mm. The largest mussel was found in the old Government Dock with a shell length of 29.7 mm and width of 17.6 mm. The growth rate decreased significantly as the mussel size increased (Figure 8).

4. **Allometric relationship:** Relationship between shell size and tissue dry weight (Figure 9, Tissue Dry Weight = 0.02 * (Shell Length) $^{2.33}$, R^2=0.955, $P < 0.01$) and Relationship between shell size and tissue dry weight (Figure 10, Shell Dry Weight = 0.06 * (Shell Length) $^{2.94}$, R^2=0.994, $P < 0.01$).

3.2. Findings in Veliger Monitoring

1. **Discovery and detection:** Quagga mussel veligers were first found in Boulder Basin; they later spread to other basins. They were detected in all basins at different depths by the end of 2007 and peaked in October of 2007. In 2008, more veligers were found in upper portions of Lake Mead than in Boulder Basin.

2. **Size and stage:** Minimum and maximum length of veligers are 77.8 µm to 355.6 µm within different stages: trochophore, straight-hinged veliger (or D-shaped veliger), umbonal veliger, and pediveliger.

3. **Settlement and distribution**: SNWA's water intake is 100 ft beneath the surface; veligers can and have settled there in an abundance comprising up to 40% of the whole zooplankton community.

3.3. Findings in Substrate Monitoring

1. **Substrate preference:** Six substrates were tested in Lake Mead: Acrylonitrile Butadiene Styrene (ABS) plastic, High Density Polyethylene (HDPE) plastic, Concrete Underlayment Board (CUB), aluminum, stainless steel and fiberglass. Although quagga mussels showed no statistically significant preference for any of these six substrates over another, the order in which mussels settled was as follows: ABS (342,483/m^2) > aluminum (293,282/m^2) > fiberglass (188,528/m^2) > HDPE (150,427/m^2) > CUB (121,645/m^2) > steel (70,121/m^2).

2. **Settlement at different depths:** Quagga mussel settlement on substrates at depths from 6-28 m was significantly greater than on substrates from 32-54 m. This divergence in settlement is likely due to the different environmental characteristics at different depths. Therefore, if materials placed in Lake Mead depths below 32 m will likely have greatly reduced mussel settlement.

3. **Growth and settlement**: More mussels were found in an ABS pipe positioned at the bottom of Lake Mead Marina (30 m below surface) than in a pipe positioned just below the surface two months after placement, and the mussel size within the surface pipe was about two times larger (1.9 mm vs. 0.8 mm) than within the deep-depth pipe.

a. Findings in Ecological Monitoring

Sentinel Island within Boulder Basin is one of the locations where quagga mussels were first found in great abundance. Significant growth of quagga mussels was also recorded in this area. Nearby monitoring station CR346.4 provided the most intensive ecological data sets before and after quagga mussel invasion. This long-term monitoring station is therefore an ideal site to provide valuable information on assessing how the invasion of quagga mussels into Boulder Basin, Lake Mead can impact the ecology of the system.

Chlorophyll *a* (Chl-*a*): The annual concentration of Chl-*a* (from surface to 6 m) during the post-quagga period (2007-2008) was only 50.6 % of that of the pre-quagga period (2000-2006) (Figure 11). Decreased nutrient loading from Las Vegas Wash was the major force reducing Chl-*a* in this area while the filtering activity of quagga mussels may have contributed to the downturn trend of Chl-*a* from 2002 to 2008 (Figure 12, analysis of covariance, $P < 0.01$).

Water transparency: The annual Secchi values for before (2000-2006) and after (2007-2008) quagga mussel invasion were 9.7 m and 10.6 m, respectively. However, no significant difference between these two periods was found. With the decreasing water elevation in Lake Mead, water clarity may have potentially increased in this station.

Dissolved oxygen (DO): The DO difference between the pre- (2000 to 2006) and post- (2007 to 2008) quagga period was not significant ($P = 0.08$) in the epilimnion (8.44 vs. 8.57 mg/L), marginally significant ($P = 0.05$) in the metalimnion (7.51 vs. 8.14 mg/L), and highly significant ($P = 0.01$) in the hypolimnion (6.56 vs. 7.43 mg/L). Increased DO in the hypolimnion could be the result of decreased primary production (Chl-*a*) in the epilimnion.

Total organic carbon (TOC): No difference in TOC was detected either in the metalimnion or in the hypolimnion (T-test, $P > 0.05$). However, in the epiliminion, TOC during post-quagga period was significantly lower than in pre-quagga period (T-test, $P < 0.05$), which should be the direct result of decreased primary production (Chl-*a*) in the epilimnion.

Nutrients: both dissolved nutrients (ammonia nitrogen and orthophosphate) and total phosphorus didn't show significant change before and after quagga mussel invasion.

Phytoplankton: Comparing to the pre-quagga period (2002-2006), there was a 23.2% increase in phytoplankton concentration and a 41.2% decrease in biovolume during the post-quagga period (2007-2008). However, neither concentration nor biovolume showed significant difference between pre- and post-quagga periods (T-test, $P > 0.05$). Tiny cybanobacteria such as *Synechococcus* are becoming more dominant in terms of concentration in Boulder Basin, but this is unlikely due to the invasion of quagga mussels. *Microcystis* was only found in December 2002, April, September, and November 2003, November 2004, August, September, and October 2005, and October 2006. No *Microcystis* was found in 2007 or 2008.

Zooplankton: In the zooplankton community, copepods, cladocerans, and rotifers didn't show significant differences before (2001-2006) of after quagga mussel (2007-2008) invasion, with exception that quagga mussel veligers were present since 2007.

Fish: The abundance of threadfin shad did not change significantly between pre- and post- quagga mussel period. Stomach analysis and stable isotope signature demonstrated that the feeding behavior of shad hasn't changed yet (Loomis 2009). Other fish such as razorback suckers have not yet been tested.

Contaminants: The concentration of mercury in quagga mussel tissue was 0.035 µg/g (dry weight). The average mercury concentration in fish from Lake Mead was 0.119 µg/g (dry weight).

Chapter 4: Current Monitoring Summary

Since the discovery of quagga mussels in Lake Mead, LMNRA, USBR, SNWA, NDOW, and CWC have actively adopted programs to monitor and minimize the impacts of these invasive organisms on Lake Mead. This chapter summarizes some of the finished and ongoing projects carried out by the different agencies.

National Park Service, Lake Mead National Recreation Area (LMNRA)

Monitoring actions: (1) Early detection of quagga mussels. All marinas were examined by divers and areas deeper than 100 feet were assessed with the aid of an ROV. (2) Transects at different depths were installed in different basins and newly designed passive samplers were installed. In total, 138 sites were sampled to estimate the density of quagga mussels in Lake Mead in 2007 (Table 2). (3) Different artificial substrates were set up in marinas to monitor the populations. Results from this work showed that Boulder Basin was the first location where quagga mussels invaded. Preliminary analysis shows that the average density in year 2007 was 505 ± 667 mussel/m^2 (N = 138). There were more mussels in rocky areas than silty areas. The density in both areas increased with depth down to approximately 21 m where the densities started to decrease as depth increased. From March to August, the density in Stewart Cliffs of Lake Mead increased 3.6 and 5.9 times at depth of 12.2 m and 18.3 m, respectively. Population size differs by location in Lake Mead. Size frequency data demonstrate that there were always three to four cohorts for each population with shell length ranging from less than 1 mm to 25 mm. The earliest length frequency analysis showed that the largest cohort in Boulder Basin was about 18.4 mm. The growth rate decreased significantly as mussel size increased (i.e., the larger the mussel, the slower the growth). The quagga mussel growth model (based on growth record in spring to early summer in 2007) in Lake Mead shows that it takes a newly hatched veliger between 2.51 and 3.16 years (depending on duration of the swimming stage before settlement) to grow to 25 mm (Moore et al. 2009). If so, the largest cohort (18.4 mm) found in February 25, 2007 must have been in Lake Mead since August 21, 2005. This growth model needs to be calibrated and validated with more field data from systematic monitoring projects. Limited data fitted by the von Bertalanffy growth equation also show that the maximum length for most mussels in Lake Mead is about 23 mm. On September 16, 2008, quagga mussel density was calculated at 54,242 individuals/m^2 for Lake Mead's South Cove, although they were not detectable in March 2007.

Control actions: (1) Inspection of incoming boats from mussel-infested states. (2) All boats should be washed with portable hot water pressure sprayers before leaving Lake Mead. Five large boat-wash facilities are being installed at marinas to facilitate this service. (3) A boat cleaning training course was offered to all marina workers, and

quagga mussel disinfection workshops were offered to concerned staff. (4) Use of the "Clean, Drain, and Dry" public message campaign (and others) to encourage boaters to prevent the spread of quagga mussels.

Sponsored research and studies: (1) Age and growth analysis of early invasive quagga mussels. (2) An ongoing quagga mussel thermal tolerance study. (3) Development of a suitable substrate device for early detection on quagga mussel (co-sponsored with SNWA). (4) Impact of quagga mussel invasion on Lake Mead shad population and diet composition. (5) Impact of quagga mussel invasion on Lake Mead benthic community. Results of ageing investigations by Robert McMahon at University of Texas, Arlington, indicate that the main invasion occurred in 2004 or perhaps 2003, which is in agreement with the previous preliminary growth model. All tested materials (high density polyethylene, white plastic, ABS black plastic, concrete underlayment board, aluminum, stainless steel, and fiberglass) for quagga mussel monitoring resulted in colonization, but colonization rate/degree depended on substrate type and depth. Depths from 10-20 m experienced 4-12 times the number of mussels settled than lower depths (Mueting et al. 2009). In three consecutive bi-monthly samplings in March, May, and July 2008, Mr. Wen Baldwin found very high recruitment on his ABS plastic pipes, with 75% or more juveniles (< 1 mm) in the whole population (an example is given in Figure 13).

NPS LMNRA also formed an *ad hoc* quagga mussel information network, the Interagency Quagga Mussel Team, with representatives from multiple agencies. A quarterly meeting serves as a platform for sharing information among multiple agencies and coping with this emergency issue in Lake Mead and other areas in the western states facing the challenge of quagga/zebra mussel invasion. The number of participants attending this meeting has grown in the past year with representation from the following agencies: 100th Meridian Initiative, Arizona Game and Fish Department, Arizona Department of Water Resources, Basic Water Company, Bureau of Reclamation, City of Henderson, Clean Water Coalition, Coachella Valley Water District, Cornell University, Desert Research Institute, Imperial Irrigation District, Lake Las Vegas Resort, Las Vegas Valley Water District, Metropolitan Water District of Southern California, National Park Service (Lake Mead), Nevada Department of Wildlife, Portland State University, San Diego County Water Authority, Southern Nevada Water Authority, University of California (Davis), University of Nevada Las Vegas, University of Nevada Reno, U.S. Bureau of Reclamation, U.S. Fish and Wildlife Service, and U.S. Geological Survey.

Southern Nevada Water Authority (SNWA)

Monitoring actions: (1) Concrete-backed boards were used in early detection of settled juveniles and their growth rates. (2) Raw water veligers from intake pumping stations were counted. (3) Veliger counts in Lake Mead weekly (Station 1-7 in Table 3). The veligers around the water intakes can reach to more than a hundred per liter (LaBounty & Roefer 2007) and sometimes can be 40% of the total zooplankton counting.

Control actions: (1) Equipment and water intake inspection by divers and facilities will be continuous until the equipment is out of service. (2) Pre-chlorination system was installed at the discharge of each intake pumping station to prevent veliger attachment to the equipment, and chlorine application before the pumping station at Intakes 1 and 2 will start in late 2008. (3) Parker Dam visit to observe USBR's coating study. (4) SNWA and MWD of Southern California went to Washington, D.C. to urge congress to invest in research related to quagga mussel eradication or management methods. SNWA and MWD sponsored an AWWARF workshop to explore strategies for responding to the presence of quagga mussels in the lower Colorado River (Zegers 2008). More detailed information about this workshop can be found in Appendix III.

Sponsored research and consultancy: (1) Risk assessment of potential impact of quagga mussels on Southern Nevada Water System drinking water plants, pumping stations, and intakes. (2) Development of suitable substrate device for early detection on quagga mussel (co-sponsored with NPS). The potential infestation risks on the water intakes are 'moderate' based on SNWA's presentation (Roefer 2008; refer to Table 4).

U.S. Bureau of Reclamation (USBR)

Monitoring actions: (1) Monthly veliger monitoring at five sites in Lake Mead, four sites in Lake Mohave, and 19 other pre-established zooplankton-monitoring stations (Station 8-28 in Table 3) with vertical plankton tows. (2) Method development for veliger identification under microscopy and polymerase chain reaction (PCR). (3). Examining the infestation status on Hoover Dam with video-enabled remotely operated vehicles (ROV). (4) Installing strainer and UV light at Hoover Dam. (5) Testing colonization risks for different anti-fouling coatings on plates. (6) Installing a self-cleaning ballast filter at Parker Dam's domestic water intake. (7) Evaluation of high-pressure spray water to clean out grates and pipelines in October 2008. (8) Assessment of plasma shock cleaning methods for hydraulic structures. (9) Initial test on controlling mussels with bacteria *Pseudomonas fluorescens*.

Control actions: (1) Strainer and UV light are installed at Hoover Dam with the strainer removing large mussels followed by treatment with UV light to kill or disable smaller mussels and prevent settlement in cooling and domestic water systems at dams. (2) Began testing 18 different coatings on plates at Parker Dam and at intakes for the Metropolitan Water District and the Central Arizona Project. (3) Self-cleaning ballast filter (50 μm) can keep all mussels and veligers from water and is being installed at Parker Dam's domestic water intake. (4) A field trial is scheduled for using high-pressure water to clean out grates and pipelines in October 2008.

Sponsored research and consultancy: (1) Risk assessment of potential impact of quagga mussels on Southern Davis Dam and Parker Dam. (2) Denver Technical Service Center and the Lower Colorado Dams Office are working to test the effects of *Pseudomonas fluorescens* (a bacteria strain discovered by Dan Molloy, New York State

Museum) in a bio-box at Davis Dam as a control measure. This study has been done with lake and river water but will not be discharged into the lake or river while more tests are needed to for US EPA approval.

Nevada Department of Wildlife (NDOW)

Actions: Since quagga mussels were discovered in NDOW's fish hatchery facilities, the program has been temporarily suspended. In 2008, NDOW examined some other inland waters within Nevada and all the test results were negative (i.e., no quagga mussels found). NDOW is concerned with tracking quagga mussel spread and finding support for minimizing the potential impacts to the Lake Mead fishery.

Clean Water Coalition (CWC)

Actions: To prevent quagga mussel infestation of SCOP pipelines, CWC is establishing a Quagga Mussel Program with MWH Global Inc. (MWH) by testing the potential infestation risks in Lake Mead.

Arizona Game and Fish Department (AZGFD)

Actions: Detection of quagga mussels and asking boater and anglers to routinely disinfect their boats.

US Fish and Wildlife Service (USFWS)

Actions: (1) Preventing the spread of aquatic invasive species through the 100th Meridian Initiative and the "Stop Aquatic Hitchhikers" national public awareness campaign. (2) Participating with Southern Nevada and California in developing the education program "Don't Move a Mussel."

Through these actions, critical baseline information on early invasion of quagga mussel into Lake Mead has been collected, programs on minimizing quagga mussel infestation on drinking water facilities and recreational facilities have been designed and successfully implemented, and a network on quagga mussels in Lake Mead has been established among multiple agencies.

Chapter 5: Suggested Monitoring
Over the Next Two Years with Existing Resources

With existing resources, the suggested monitoring programs over the next two years include quagga mussel monitoring, water quality monitoring, and fish and invertebrates monitoring as described within this chapter. Appendix V documents standardized protocols, existing or proposed, for monitoring.

5.1. Quagga mussel monitoring

Baseline information on quagga mussel veligers and adults has been collected in the past two years. To establish a cost-effective, long-term, and scientifically sound quagga mussel monitoring plan, it is recommended that the following programs be established or continued.

5.1.1. Adult and juvenile monitoring

Transects will be set up in different areas with different substrates with focus on Boulder Basin. Seven transects are in Boulder Basin with quarterly sampling: CR346.4, LVB 7.3, LVB 3.5, CR351.7, Sentinel Island, Black Island, and Boulder Island. Five other transects are sampled annually: Stewart Cliffs in Virgin Basin, Cormorant Point in Overton Arm, The Temple in Temple Basin, Sandy Point in Gregg Basin, and Tequila Cove between Lakes Mead and Mohave. More detailed information on sampling locations is listed in Table 5 and Figure 15 and detailed sampling protocols are provided in Appendix V.

5.1.2. Quagga mussel veliger monitoring

Regular veliger monitoring by SNWA and USBR should continue. This includes SNWA's seven weekly regular zooplankton-monitoring stations, USBR's four monthly veliger- and 19 monthly zooplankton-monitoring stations (Table 3 and Figure 16). The Sentinel Island station sampled by UNLV needs to be used for investigating the veliger abundance at different depths. More detailed information on veliger-sampling locations is listed in Table 3 and detailed sampling protocol is listed in Appendix V.

5.1.3. Contamination monitoring

Like other bivalves, quagga mussels can bioaccumulate contaminants (e.g., Hg, Se, PCBs, PAHs) in their tissue and transfer these contaminants to higher trophic levels, such as some species of ducks and fish in Lake Mead. The concentration of mercury in fish was about three times that of quagga mussel tissue. The potential for bioaccumulation is evident. As such, quagga mussels are ideal biomarkers for contaminants. Sediment and water concentrations of mercury are very low and difficult to detect, but tissue concentrations are within the range for simple analytical methods (Mueting 2009). Monitoring of the contaminant transfer from quagga mussels to fish and waterfowl is recommended.

5.1.4. Substrate monitoring

Substrate monitoring needs to be set up along a transect of Las Vegas Bay from Las Vegas Wash to Boulder Basin. Quagga mussels were first found in Boulder Basin; from there they spread exponentially and grew quickly. However, there is no sign that they have been similarly successful in the inner Las Vegas Bay. Is this due to waste water from Las Vegas Wash, extremely high nutrients, other toxic compounds, or a particular mixture of different compounds? Alternatively, is it due to other factors, such as substrate type, rich organic materials in the sediment, or a too high phytoplankton biomass with less edible species? If lower survival and growth are results from the characteristics of the wastewater from Las Vegas Wash, the future SCOP project may help mitigate quagga mussels at the bottom of Lake Mead by impacting their survival, settlement, and growth. The substrate monitoring set by CWC at the bottom and surface of Lake Mead Marina should be continued. Substrates set by NPS volunteer Wen Baldwin needs to be continued to monitor settlement, growth, and life history of quagga mussels.

5.2. Water quality monitoring

In Boulder Basin, Lake Mead, SNWA, USBR, and CWC's BBAMP have 15, 7, and 5 permanent stations to study water quality and limnology. These parameters include, but not limited, temperature, dissolved oxygen, conductivity, pH, Secchi depth, turbidity, alkalinity, total calcium, biological oxygen demand (BOD), chemical oxygen demand (COD), orthophosphate phosphorus, total phosphorus, ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, Kjeldahl nitrogen, total nitrogen, bromide, perchlorate, selenium, mercury, PCBs, total organic carbon, fecal coliform bacteria (especially *E. coli*), chl-*a*, phytoplankton biomass and species composition, and zooplankton, with sampling frequency from weekly and biweekly, to monthly and bimonthly and sampling depth from surface to hypolimnion depending on each parameter and on the needs of each agency. The long-term record of these

multiple parameters and future data collection provide excellent support for how quagga mussel can affect these water quality parameters. The following parameters are suggested to be shared with the long-term quagga mussel monitoring plan: (1) Chl-*a*; (2) Secchi depth and/or turbidity; (3) Orthophosphate and total phosphorus; (4) Ammonia and nitrate nitrogen; (5) total organic carbon; (6) dissolved oxygen; (7) phytoplankton (concentration and biovolume); and (8) zooplankton.

5.3. Monitoring Fish and Invertebrates

Threadfin shad (*Dorosoma petenense*) is important to the Lake Mead fishery as the primary prey of striped bass (*Morone saxatilis*); monitoring of this fish by NDOW should be continued to see if there is any change that threatens the sport fishery. Endangered species razorback suckers (*Xyrauchen texanus*) are in extreme danger in Lakes Mead and Mohave as quagga mussels are degrading their habitat. Long-term datasets are necessary to track how quagga mussels impact fisheries in the Lake Mead ecosystem.

Assessment of benthic ecology of Lake Mead during the early invasion of quagga mussels should be continued to document long-term benthic invertebrate composition, abundance, and production along different depth gradients in the lake.

5.4. Infrastructure Maintenance and Public Education

Quagga mussel monitoring on boat, water intakes, and dams should be continuous. Boat inspections, boat cleaning training events, and use of public messaging campaigns and other outreach activities are encouraged. Regular cleaning and measures for water intakes and dams should be taken.

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Chapter 6: Tables, Figures, Maps, and Charts

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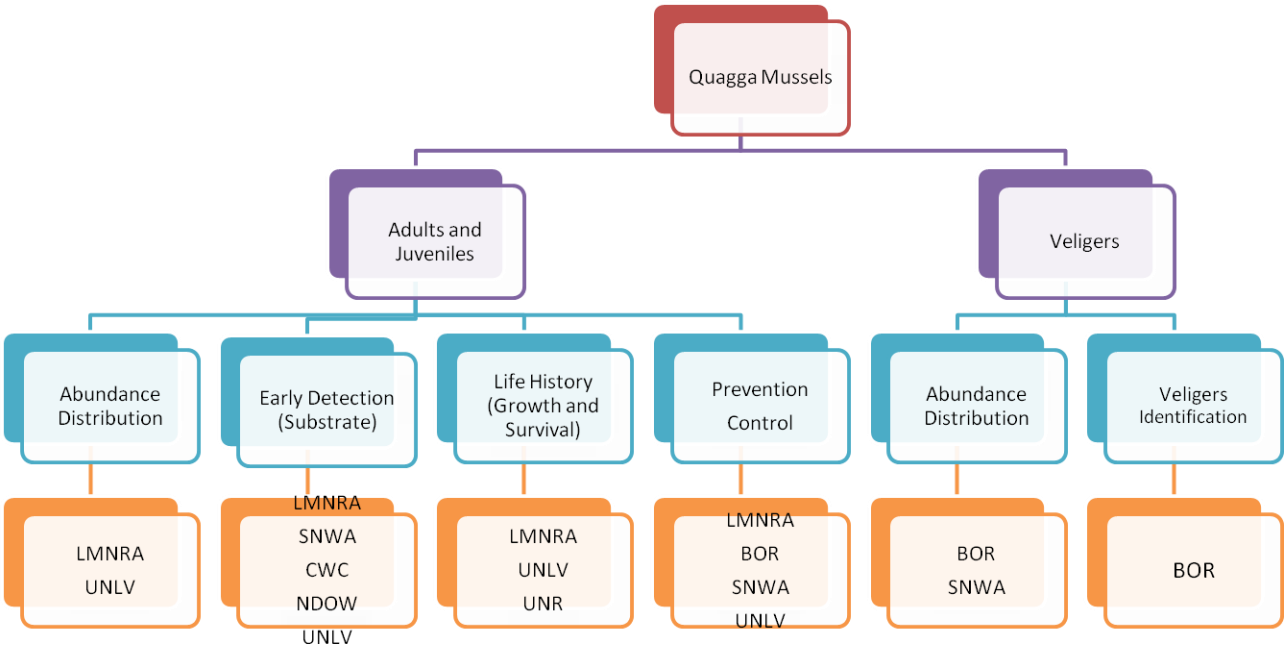

Figure 1. Current quagga mussel monitoring activity

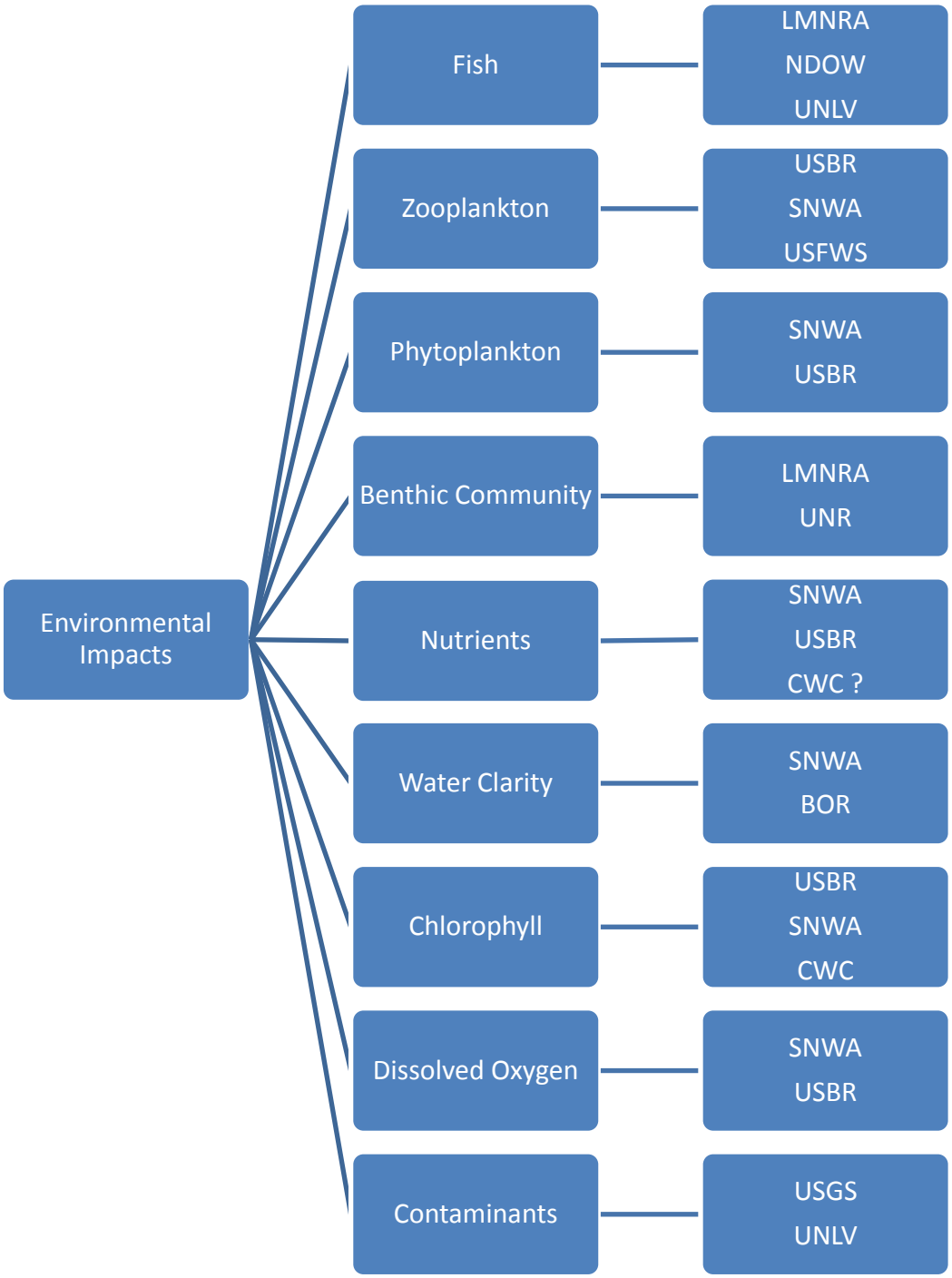

Figure 2. Existing environmental monitoring of impacts of quagga mussels on Lake Mead

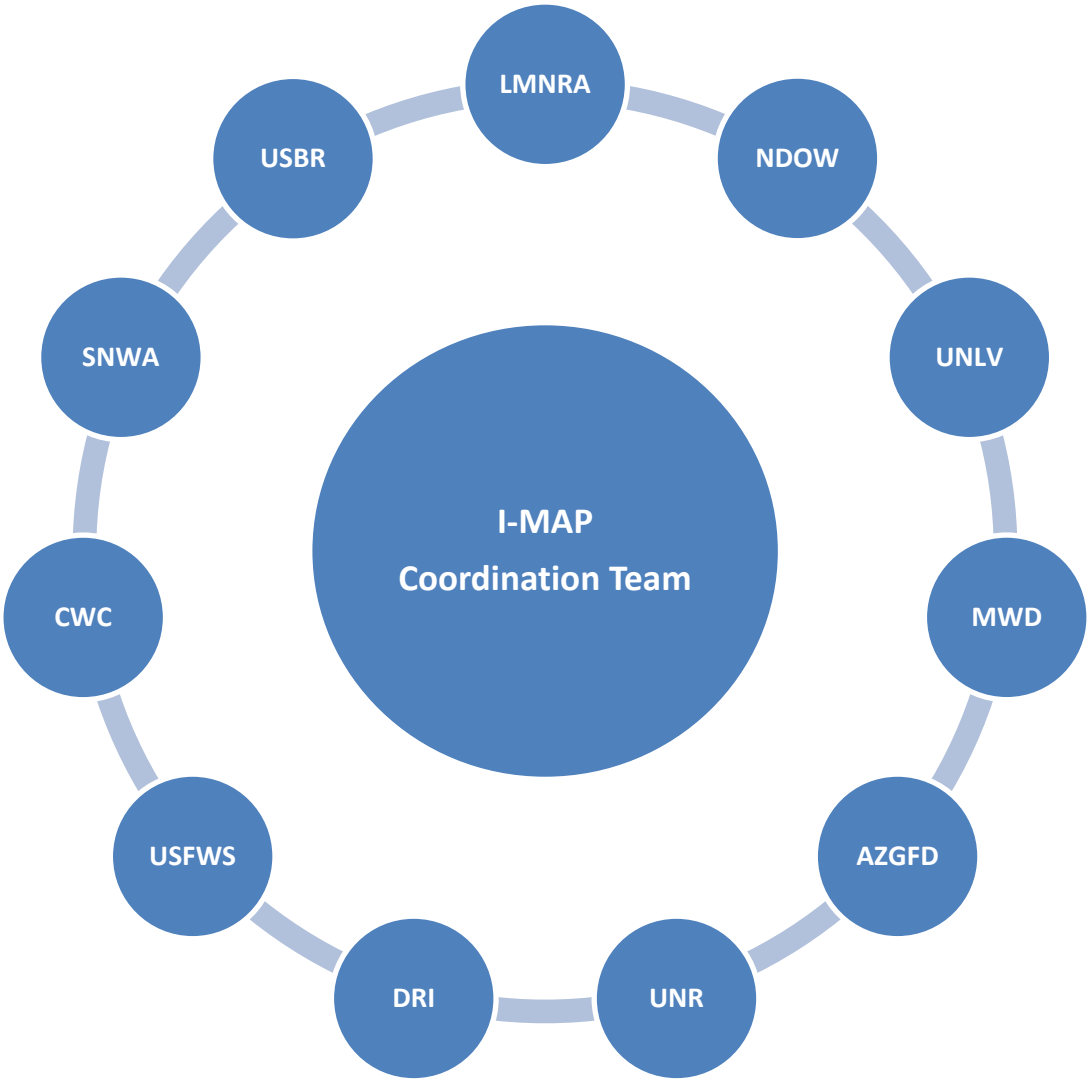

Figure 3. I-MAP team members

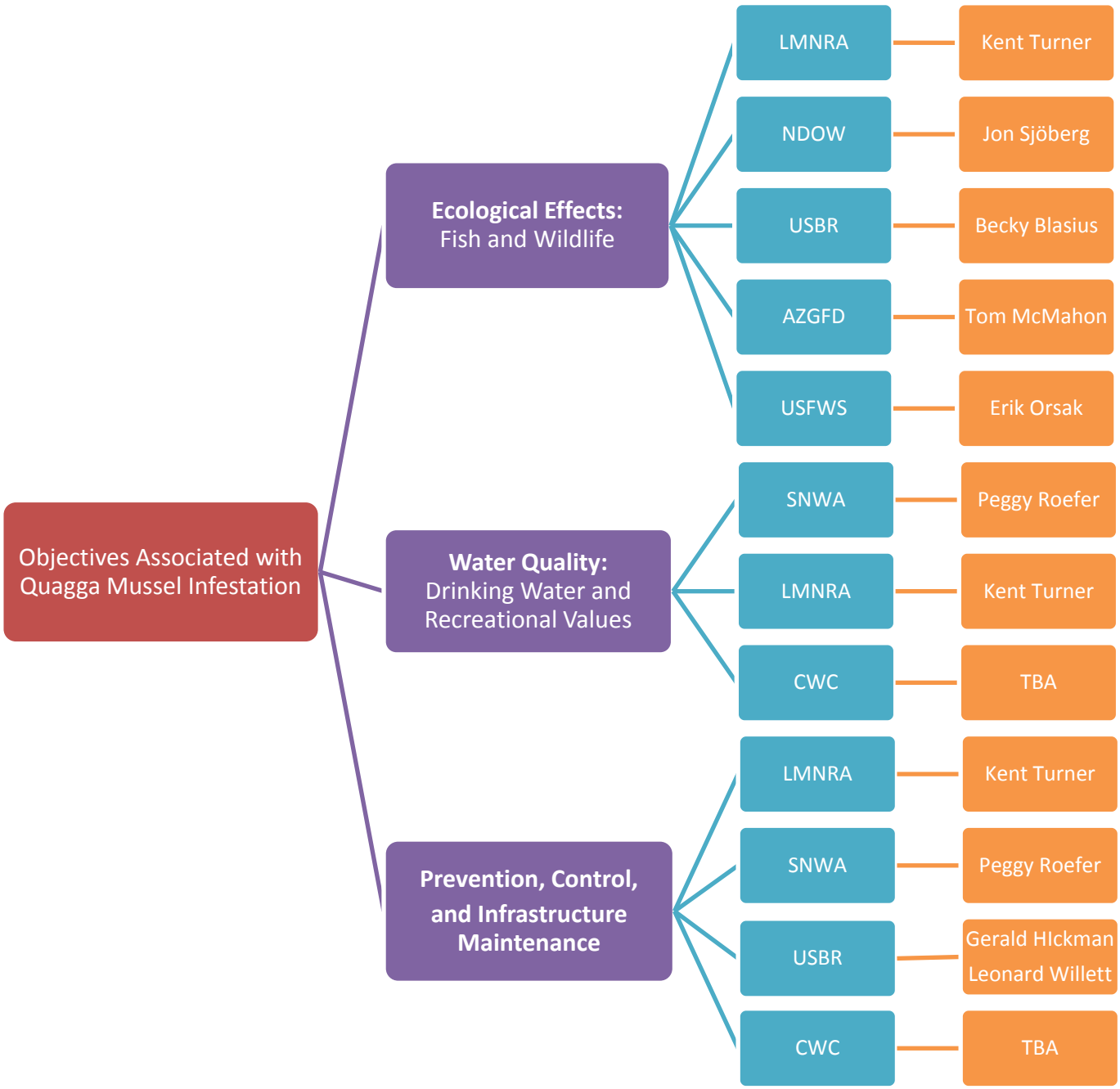

Figure 4. Interagency representatives for I-MAP objectives

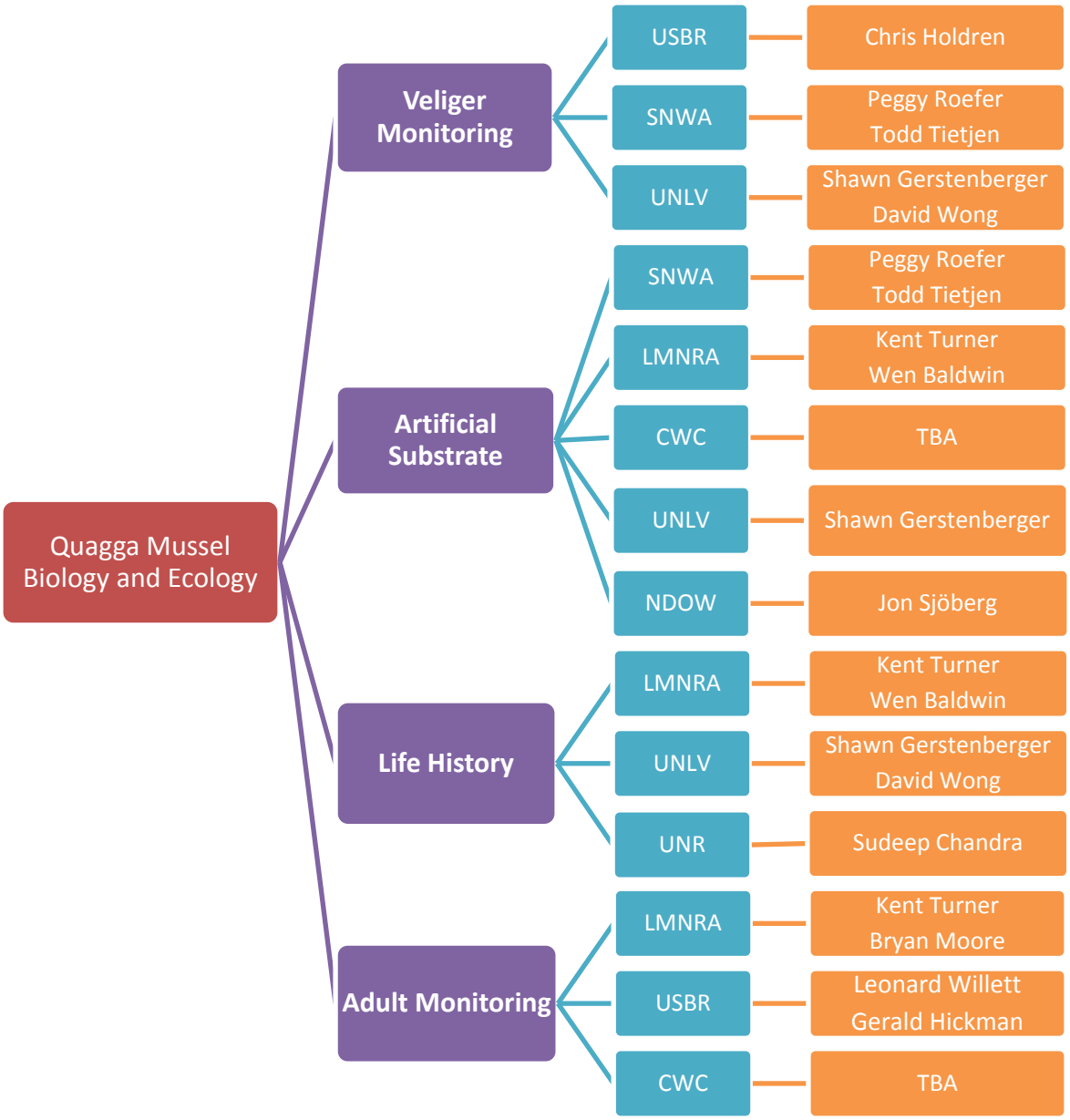

Figure 5. I-MAP representatives for quagga mussel monitoring

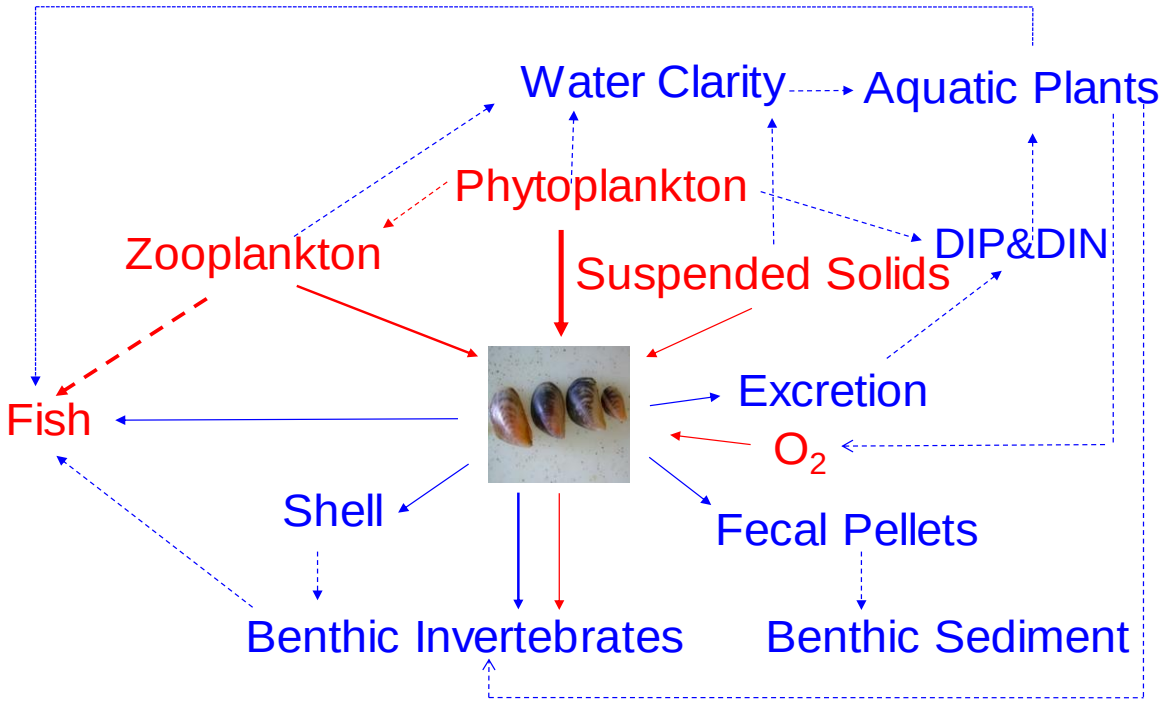

Figure 6. Potential ecological impacts of quagga mussels on the Lake Mead ecosystem (Wong & Gerstenberger 2009). Negative and positive effects are shown in red and blue, respectively. Solid and dashed lines represent direct and indirect impacts, respectively. The wider the line, the more serious the impact.

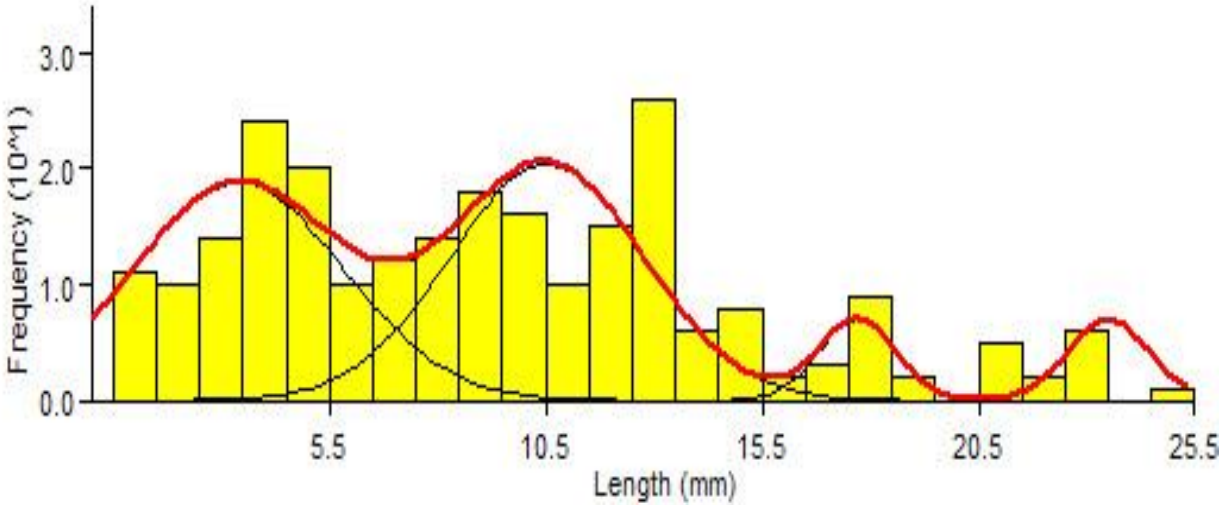

Figure 7. Length frequency of quagga mussels in Black Island, Lake Mead (March 26 2007)

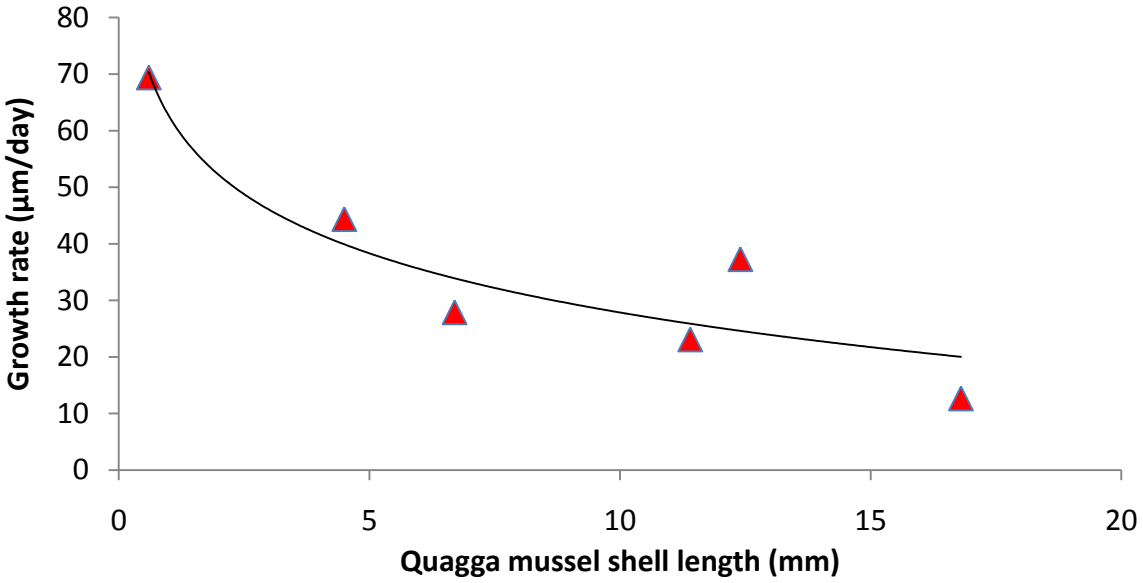

Figure 8. Growth rate of quagga mussels of different sizes in Lake Mead

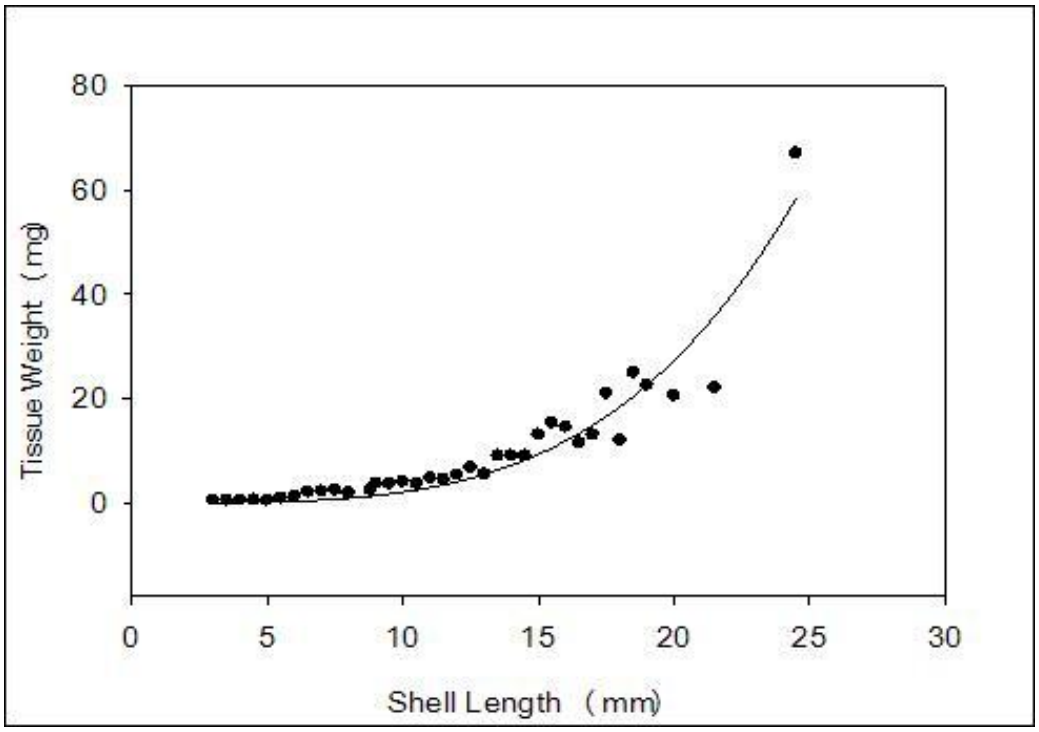

Figure 9. Relationship between shell size and tissue dry weight (Tissue Dry Weight = 0.02 * (Shell Length) $^{2.33}$, R^2=0.955, P < 0.01).

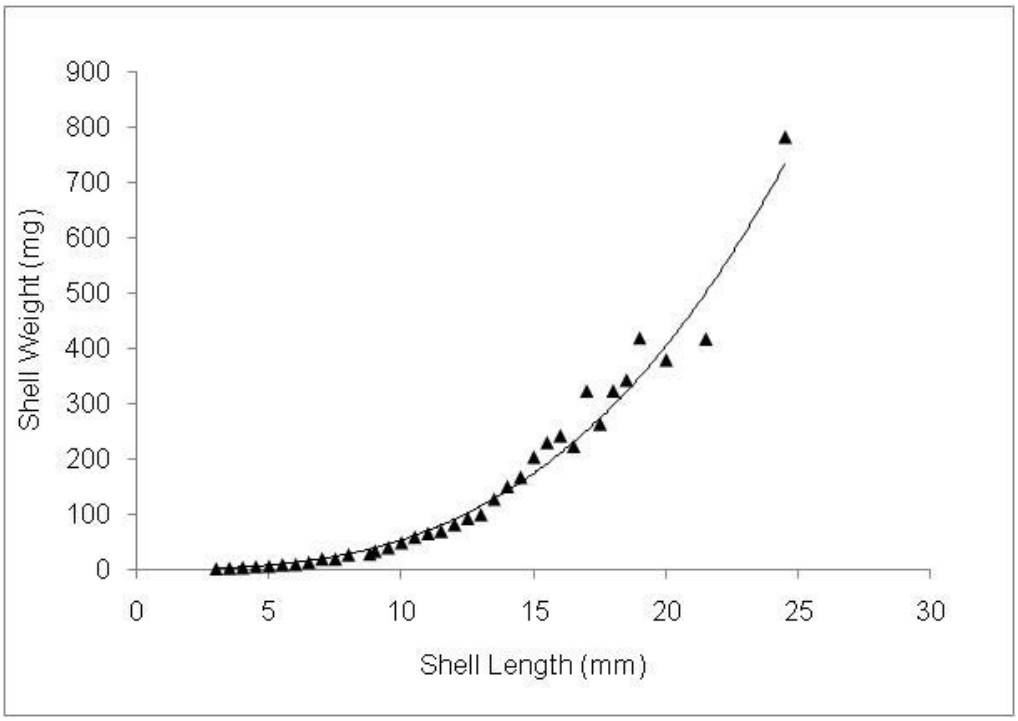

Figure 10. Relationship between shell size and tissue dry weight (Shell Dry Weight = 0.06 * (Shell Length) $^{2.94}$, R^2=0.994, P < 0.01).

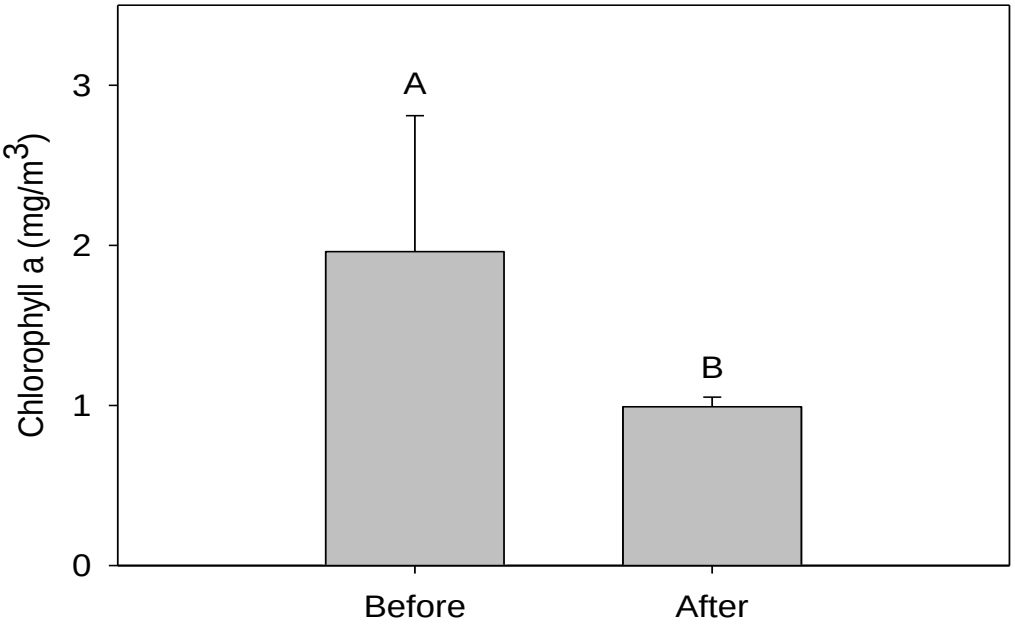

Figure 11. Annual Chlorophyll *a* concentration of Boulder Basin (based on CR346.4) before (2000-2006) and after (2007-2008) quagga mussel invasion.

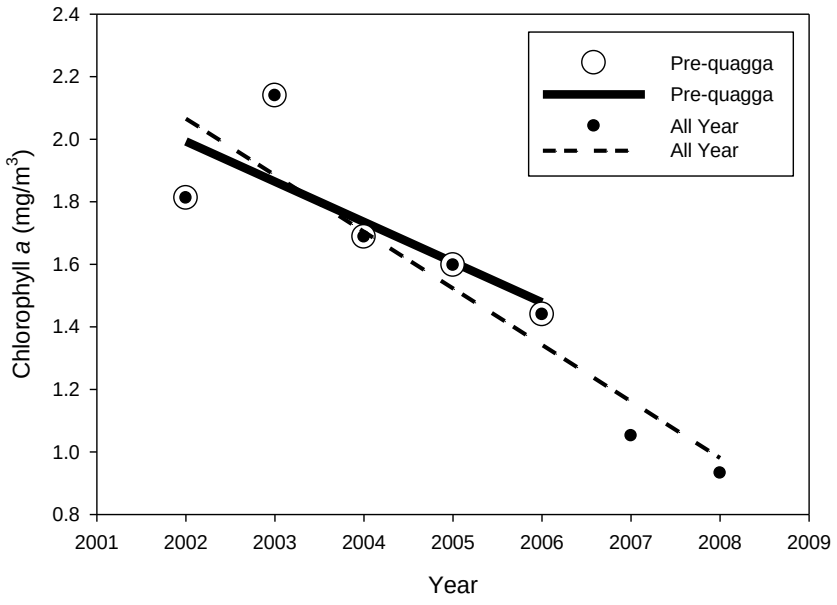

Figure 12. Temporal trend of annual Chlorophyll *a* concentration of Boulder Basin (Pre-quagga (2002-2006): Y = 259.7 – 0.1287 X (R^2 = 0.60); All Year (2002-2008): Y = 364.13 – 0.1813 X (R^2 = 0.85). Y-axis represents Chlorophyll *a* and X-axis represents the year).

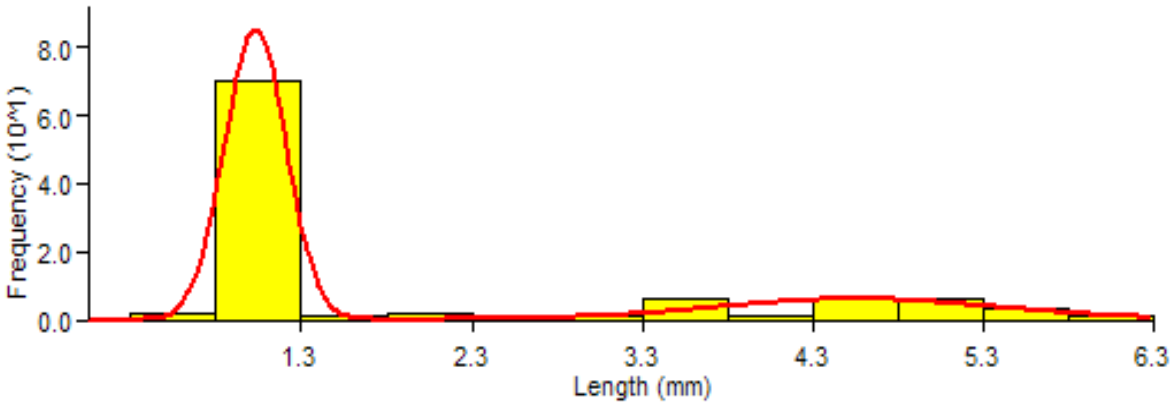

Figure 13. Quagga mussels in an artificial substrate monitoring station in Las Vegas Boat Harbor Marina on March 19, 2008.

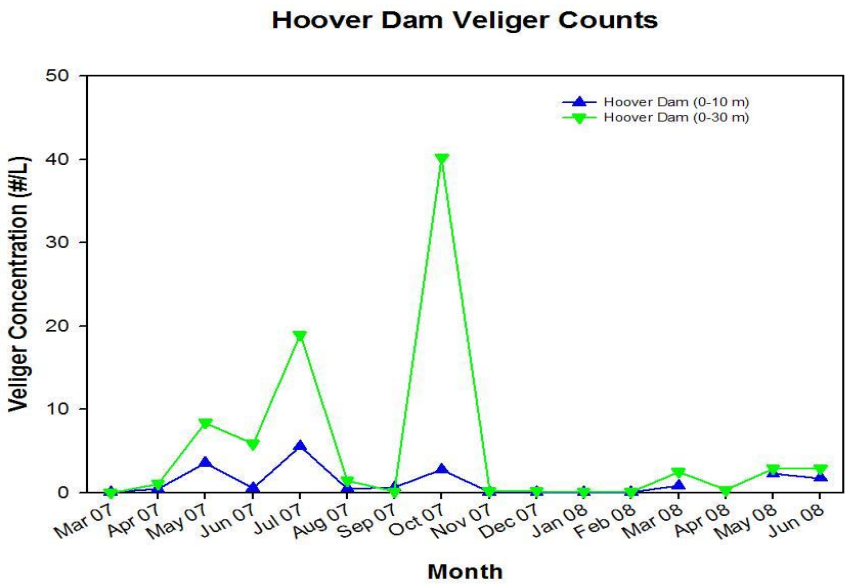

Figure 14. Quagga mussel veliger concentration in Hoover Dam at two depths (Holdren 2008a).

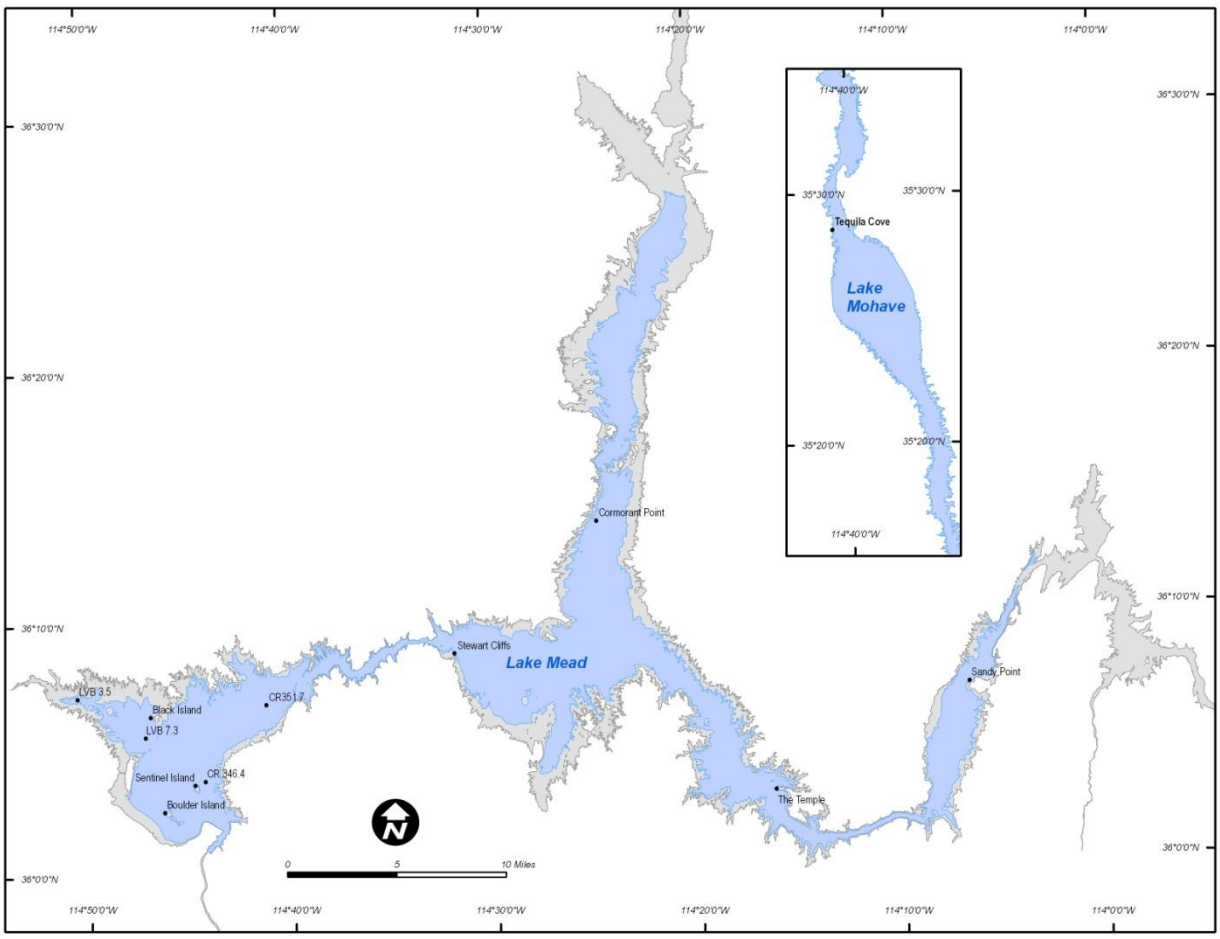

Figure 15. Sampling stations for adults and jeveniles.

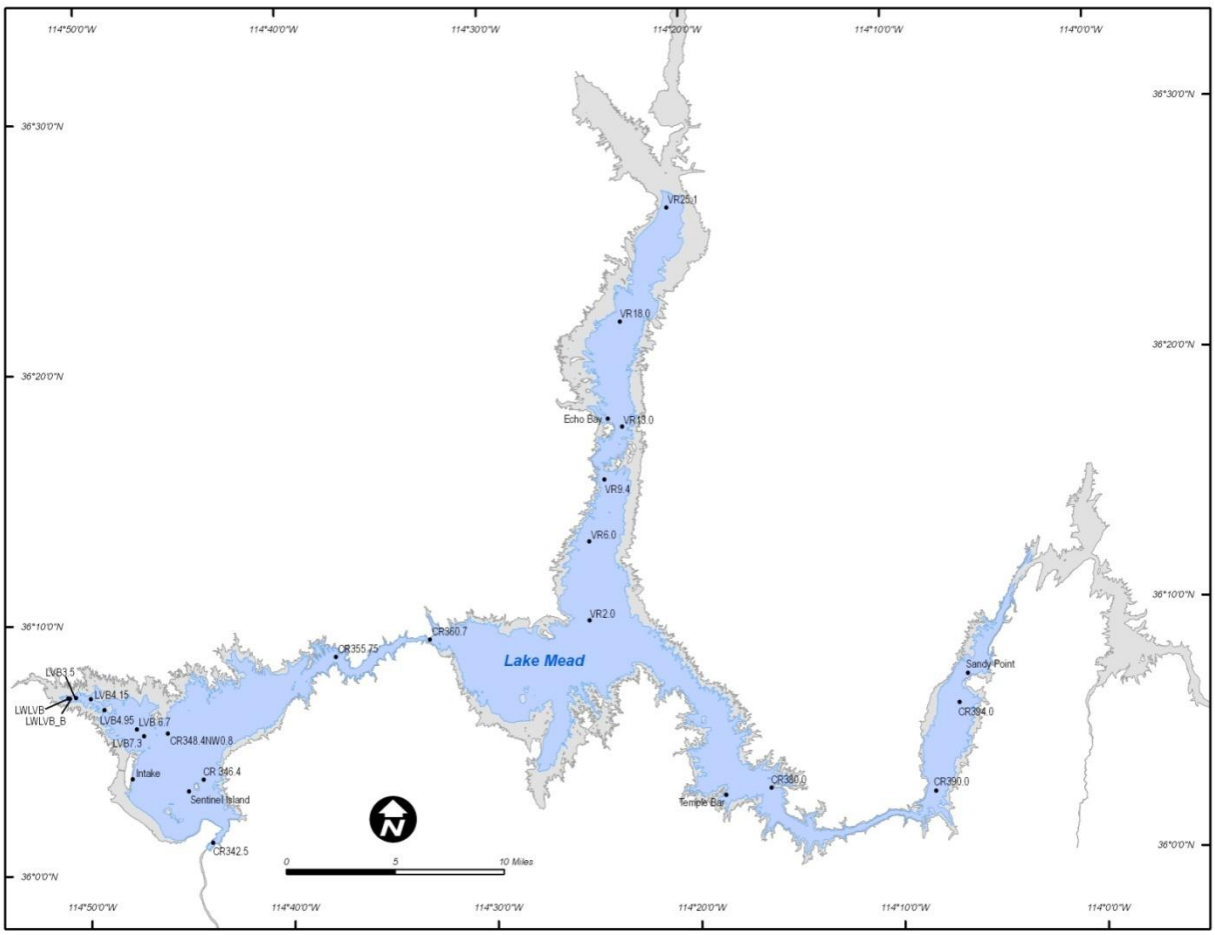

Figure 16. Sampling stations for quagga mussel veligers.

Table 1. Expenditures of four federal agencies in fiscal year 2008 to deal with quagga mussels (Wirkus 2008).

Agency	Expenditure	Purpose
USBR	$800,000	Research on issues related to quagga mussels
NPS	$5,000,000	Inspection
USGS	$200,000	Support to deal with quagga mussels
USFWS	$1,800,000	Aquatic Invasive Species Program in the Western States

Table 2. Quagga mussel monitoring by NPS in 2007.

Basin/Lake	Station Name	Latitude	Longitude
Boulder Basin	Sentinel Island	36° 03' 34"	-114° 44' 48"
Boulder Basin	Black Island	36° 06' 18"	-114° 46' 56"
Boulder Basin	Boulder Island	36° 02' 30"	-114° 46' 20"
Virgin Basin	Stewart Cliffs	36° 05' 09"	-114° 19' 10"
Overton Arm	Cormorant Point	36° 08' 14"	-114° 14' 53"
Temple Basin	The Temple	36° 01' 43"	-114° 09' 46"
Gregg Basin	Sandy Point	36° 06' 58"	-114° 06' 41"
Lake Mohave	Tequila Cove	35° 17' 08"	-114° 24' 26"

Table 3. Quagga mussel veligers monitoring stations in Lake Mead.

Station	Station Name	Latitude	Longitude	Agency	Smpling Frequency	Sampling Mode
1	LWLVB	36° 07' 04.19" N	114° 50' 56.65" W	BOR/SNWA	Weekly	Zooplankton
2	LWLVB_B	36° 07'0 3.58" N	114° 50' 51.25" W	SNWA	Weekly	Zooplankton
3	LVB4.15	36° 07' 00.82" N	114° 49' 49.50" W	BOR/SNWA	Weekly	Zooplankton
4	LVB 6.7	36° 05' 46.03" N	114° 47' 35.27" W	SNWA	Weekly	Zooplankton
5	INTAKE	36° 03' 47.05" N	114° 47' 51.32" W	SNWA	Weekly	Zooplankton
6	CR 346.4	36° 03' 41.96" N	114° 44' 20.88" W	BOR/SNWA	Weekly	Zooplankton
7	CR348.4NW0.8	36° 05' 34.85" N	114° 46' 03.97" W	SNWA	Weekly	Zooplankton
8	LVB3.5	36° 07' 04.92" N	114° 50' 33.42" W	BOR	Monthly	Zooplankton
9	LVB4.95	36° 06' 34.42" N	114° 49' 10.66" W	BOR	Monthly	Zooplankton
10	LVB7.3	36° 05' 29.39" N	114° 47' 14.76" W	BOR	Monthly	Zooplankton
11	CR342.5	36° 01' 09.78" N	114° 43' 57.59" W	BOR	Monthly	Veliger/Zooplankton*
12	VRLM	Variable		BOR	Monthly	Zooplankton**
13	MRLM	Variable		BOR	Monthly	Zooplankton**
14	VR25.1	36° 26' 06.90" N	114° 20' 48.07" W	BOR	Monthly	Zooplankton
15	VR18.0	36° 21' 36.09" N	114° 23' 15.15" W	BOR	Monthly	Zooplankton
16	VR13.0	36° 17' 23.74" N	114° 23' 17.07" W	BOR	Monthly	Zooplankton
17	VR9.4	36° 15' 18.17" N	114° 24' 13.20" W	BOR	Monthly	Zooplankton
18	VR6.0	36° 12' 50.34" N	114° 25' 03.28" W	BOR	Monthly	Zooplankton
19	VR2.0	36° 09' 41.01" N	114° 25' 08.83" W	BOR	Monthly	Zooplankton
20	CRLM_A	Variable		BOR	Monthly	Zooplankton
21	CR394.0	36° 06' 00.70" N	114° 07' 00.51" W	BOR	Monthly	Zooplankton
22	CR390.0	36° 02' 29.59" N	114° 08' 17.24" W	BOR	Monthly	Zooplankton
23	CR380.0	36° 02' 48.30" N	114° 16' 24.09" W	BOR	Monthly	Zooplankton
24	CR360.7	36° 09' 05.11" N	114° 33' 02.48" W	BOR	Monthly	Zooplankton
25	CR355.75	36° 08' 28.97" N	114° 37' 41.66" W	BOR	Monthly	Zooplankton
26	Eco Bay	36° 17' 44.30" N	114° 23' 58.78" W	BOR	Monthly	Veliger
27	Temple Bar	36° 02' 33.36" N	114° 18' 38.34" W	BOR	Monthly	Veliger
28	Sandy Point	36° 07' 10.02" N	114° 06' 33.37" W	BOR	Monthly	Veliger
29	Sentinel Island	36° 03' 14.50" N	114° 45' 05.40" W	UNLV	Weekly	Veliger***

*Apart from regular zooplankton sampling, extra samples from 0-10 m and 0-30 m are collected for veliger counting only;

** These two sites are being monitored but these two will be combined as one if water level falls below approximately 1110 ft;

* **Multiple depths will be smapled at different depth (surface water, 0-5 m, 5-10 m, 10-20 m, 20-30 m, 30-40 m, 40-50 m, 50-60 m);

Table 4. Quagga mussel infestation risk on water intakes by RNT Consulting

Variable	IPS-1	IPS-2	Risk Potential
Alkalinity, total mg $CaCO_3$/L	136.8	136.5	Intense
Calcium, mg/L	80.3	74.8	Intense
Chlorophyll-*a*, μg/L	1.4	<1.4	No infestation
pH	8	8	Moderate
Dissolved Oxygen, mg/L	7 - 8	7 - 8	Moderate
Summer Temperature, °C	17	15	Moderate
Total phosphorus, μg/L	6.1	2.9	Little to none
Overall likelihood of water quality supporting a mussel infestation:			**Moderate**

Table 5. Sampling location and frequency of adult and juvenile quagga mussels in Lakes Mead and Mohave.

Transect	Station Name	Latitude	Longitude	Substrate	Sampling	Frequency
1	CR 346.4	36° 03' 42" N	114° 44' 21" W	Soft	Grab	Quarterly
2	LVB 7.3	36° 05' 29" N	114° 47' 15" W	Soft	Grab	Quarterly
3	LVB 3.5	36° 07' 05" N	114° 50' 30" W	Soft	Grab	Quarterly
4	CR351.7	36° 06' 42" N	114° 41' 14" W	Soft	Grab	Quarterly
5	Sentinel Island	36° 03' 34" N	114° 44' 48" W	Hard	Diving	Quarterly
6	Black Island	36° 06' 18" N	114° 46' 56" W	Hard	Diving	Quarterly
7	Boulder Island	36° 02' 30" N	114° 46' 20" W	Hard	Diving	Quarterly
8	Stewart Cliffs	36° 05' 09" N	114° 19' 10" W	Hard	Diving	Annual
9	Cormorant Point	36° 08' 14" N	114° 14' 53" W	Hard	Diving	Annual
10	The Temple	36° 01' 43" N	114° 09' 46" W	Hard	Diving	Annual
11	Sandy Point	36° 06' 58" N	114° 06' 41" W	Hard	Diving	Annual
12	Tequila Cove	35° 17' 08" N	114° 24' 26" W	Hard	Diving	Annual

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Chapter 7: Desired future monitoring

The suggested ongoing monitoring program will shed light on quagga mussels from biological and ecological perspectives. However, there are still a lot questions that remain to be answered (Appendix IV). If the program has the opportunity to expand, the following monitoring projects should be included in the I-MAP quagga mussel-monitoring program. The answer can provide strategic advice to lake managers for making long-term policies to evaluate this large-scale biological invasion and maintain the water quality for millions of people in the lower Colorado River Basin.

1. **Filtration rates of quagga mussels:** although there are filtration data available for quagga mussels in temperate waters, it is not known how filtration rate differs in this warm water reservoir or at different seasons therein. Filtration rate is an important factor to consider in assessing how much and in what timeframe these mussels can impact the Lake Mead ecosystem.

2. **Reproductive behavior of quagga mussels:** quagga mussels have been very successful at reproduction in Lake Mead due to the favorable environmental conditions it provides this species. For example, in South Cove, the density of quagga mussels reached 54,000 individuals per square meter from non-detectable 1.5 years ago. However, there is no systematic study on the reproductive state and recruitment rate of quagga mussels in the lower Colorado River, such as Lake Mead, though it is estimated that these mussels reproduce multiple times a year. By understanding the reproductive behavior of quagga mussels, we can explain how environmental variables such as temperature, food, and water velocity can affect recruitment in Lake Mead and make predictions about future colonization.

3. **Substrate monitoring for all inland water bodies in Nevada:** since we have established a successful substrate-monitoring program for Lakes Mead and Mohave, this program should be implemented for other bodies of water within Nevada for early detection of quagga mussel veligers.

4. **Impact of quagga mussels on water quality:** Water chemistry and phytoplankton composition can be changed by quagga mussels by their selective feeding behavior (rejection of cybanobacteria and preference for green algae) and excreting phosphorus and nitrogen. Though no significant change has yet been observed in the past two years, the potential for change is still high.

5. **Impact of quagga mussel treatment on drinking water quality:** Lake Mead is the primary drinking and industrial water source for Las Vegas and millions of people living in the lower Colorado River Basin. Invasive quagga mussels are clogging drinking water pipes, intakes and other infrastructure. The damage is destructive and profound. To deal with these invasive pests, chlorination is the only licensed option for drinking water in USA. However, chlorination will result in a production of carcinogenic byproducts, trihalomethanes (THMs).

For example, Sothern Nevada Water Authority has treated our drinking water to deal with quagga mussel problems for their pipes with chlorine since 2007. Due to the continuous usage of chlorine, THMs in the drinking water is becoming a concern of environmental health as the concentration of THMs in 2010 (68 ppb) is closer to the U.S. EPA drinking water safety threshold. A more sustainable alternative treatment should be studied and implemented.

6. **Carrying capacity of quagga mussel in Lake Mead:** Although quagga mussels are extraordinarily successful in Lake Mead, there is always some factor that can finally limit the carrying capacity, such as space, food, disease, or other physical and/or chemical factor. Among all these factors, food could be the most likely one to limit the growth of quagga mussels in the near future because it is currently just in the margin of the threshold. If we can find out the critical food concentration threshold at which quagga mussels cannot grow, it is possible to estimate the carrying capacity of quagga mussels in Lake Mead and even predict when they will reach it.

Appendix I Life History of Quagga Mussels

Like marine bivalve mollusks, quagga mussels have two life forms: planktonic and benthic. After external fertilization between a mature egg and a sperm cell in the water column, embryological development occurs as a single cell divides by mitosis. There are three major stages in the quagga mussel life cycle: larval, juvenile, and adult. Larvae are planktonic, free-swimming veligers and juveniles and adults are mostly motile individuals attaching to substrates with their proteinaceous byssal threads. Usually, the planktonic larval stage is further divided into four periods: trochophore; straight-hinged veliger (or D-shaped veliger); umbonal veliger; and pediveliger. During the pediveliger period, veligers swim using a velum (propulsion organ), crawl by means of foot, and they secrete proteinaceous byssal threads to settle on a substrate. The amount of time required for a fertilized egg to develop into a fully developed juvenile can range from eight to 240 days, which is dependent on many environmental factors, such as temperature, food quality and quantity, and available substrates (Nichols 1996). Most veligers appear to settle on appropriate substrates 18 to 90 days after fertilization (Ackerman et al. 1994, Crosier & Molloy 2001). After metamorphosis, pediveligers become juveniles. Most mussels become sexually mature when they grow to 10 mm (exceptions for those less than 10 mm also occur).

Adults generally can live for three years in temperate climates (lifespan may be different in Lake Mead because the water is warmer). In June 2008, it was reported that dead adult mussels were observed in Rufus Cove, above and below thermocline, by NPS divers Bryan More and Ross Haley, and in Echo Bay by NPS volunteer Wen Baldwin, and in Las Vegas Boat Harbor, Government Dock, Boulder Beach, and Lake Mead Marina by Melissa Cheung (Personal communications). These mussels may be around 2.5 years old. Natural mortality was also found in mussels attached to ABS plastic substrates in the Las Vegas Boat Harbor. Some dead mussels are less than a year old (10 mm or less in shell length) and colonized with each other in high densities (> 100,000 individuals/m^2). The physical crowding usually has negative impacts on feeding, food availability, and body condition of bivalves (Senechal et al. 2008).

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Appendix II Boulder Basin Adaptive Management Plan (BBAMP): Items of Concern (IOCs)

The Clean Water Coalition (CWC) comprises all permitted municipal wastewater dischargers into the Las Vegas Wash (and from there to Lake Mead). Members (City of Henderson Water Reclamation Facility, the Clark County Water Reclamation District, and the City of Las Vegas Water Pollution Control Facility) established the CWC to implement the Systems Conveyance and Operations Program (SCOP) project to improve the manageability of the effluent discharged to the Colorado River system. The SCOP project will develop a deep-water outfall effluent pipeline in the Boulder Basin of Lake Mead. To ensure that project purposes of maintaining existing high water quality of Lake Mead are met, the SCOP process developed the Boulder Basin Adaptive Management Plan (BBAMP), managed by the CWC, to establish the baseline conditions and management of the operations of the wastewater treatment and the SCOP facilities while maintaining the water quality as drinking water resource, the ecosystem health, and recreational value of Boulder Basin of Lake Mead. It also recognizes the need for identifying and managing non-effluent related stressors in the Lake Mead ecosystem. The SCOP program is developed, managed, and implemented by the SCOP Core Management Team (CMT) and become the basis for the operation of SCOP facilities (Karafa et al. 2006). The CMT consists of agencies with administrative and operational responsibilities for the use and management of water and water quality in Lake Mead. All the team members have the delegated authority to represent agency decisions and the ability to commit resources under local agency control within statutory limitations; they are CWC, SNWA, NPS, and USBR. It is recommended that the CMT establish a standing technical coordination committee that will consist of federal, state, and local agencies that have a participating interest in the water use and water quality of Lake Mead. The agencies appointed to a committee will provide technical, logistical, and if necessary, funding to accomplish specialized activities. The BBAMP process has recently outlined five Items of Concerns (IOCs) and quagga mussels are constituents of all these IOCs.

1. **Drinking Water Protection: specifics include (1) disinfection byproducts (DBPs) such as bromate, THMs (Trihalomethanes) and NDMA (N-Nitrosodimethylamine) that are formed from precursors such as bromide, total organic carbon, and organic nitrogen, (2) pharmaceuticals and personal care products, (3) pathogens, (4) algal toxins, and (5) salinity.**

   Evaluation actions are:

   1. SNWA measures DBPs, precursors, TOC, bromide at SNWA intakes and Hoover Dam outlet. MWD measures the same at Whitsett (ongoing weekly and monthly monitoring);
   2. Analyze DBP formation potential (MWD and SNWA periodic studies);
   3. Monitor contaminants (SNWA measures quarterly in LV Wash and Lake mead, and MWD conducts periodic studies in Colorado River system);

4. Analyze NDMA presence and potential (SWNA and MWD periodic studies);
5. Analyze impacts of constituents on SNWA and downstream drinking water supplies (SNWA piloting impacts of bromide, TOC, and temperature);
6. Analyze trace organics in Fathead Minnow study and CCWRD Ozone/Ultrafiltration pilot;
7. Monitor constituents and flow in dry and wet weather from LV Wash, SCOP, Colorado River, Muddy River and Virgin River (ongoing monitoring by CWC, CCRFCD, SNWA, BOR, USGS);
8. Monitor Lake Mead currents and stratification (ongoing by SNWA, USBR, CWC);
9. Evaluate salinity sources;
10. Understand existing source water protection programs;
11. Compare water quality with state and federal safe drinking water standards;
12. Identify sources and events that cause problem levels of constituents under changing lake level;
13. Study effluent from different wastewater treatment techniques (ongoing monitoring of Se, TDS, TOC, Bromate and Bromide by CWC members);
14. Develop standardized list of analytes.

2. Phosphorus (P) in Lake Mead and below Hoover Dam: Specifics are (1) P contributing to the growth of algae in reservoirs, and (2) P contributing to the productivity of fisheries.

Evaluation actions are:

1. Measure effluent loading and variations (CWC members) (continuous data is from 1992 but could be as early as 1970s);
2. Understand differences in P among water reclamation facilities (CWC members);
3. Understand variable or seasonal loading from storm water and dry weather urban flows (CCRFCD, SNWA, COH);
4. Measure P in Colorado, Muddy and Virgin Rivers entering Lake Mead, the Las Vegas Wash entering Las Vegas Bay, and in the Colorado River downstream of Hoover Dam (BOR, USGS, CLV);
5. Measure N and P in reservoir profiles (CLV BBAMP and compliance monitoring, USGS);
6. Calculate the P budget of Lake Mead, particularly Las Vegas Bay and Boulder Basin (ELCOM/CAEDYM model);
7. Measure chlorophyll in Lake Mead (CLV BBAMP and compliance monitoring);
8. Evaluate quagga mussels and responses (USBR, NPS, CLV, Water 2025);
9. Incorporate measurements of nitrogen (N), phosphorus (P) and temperature (T) in the Colorado River from Hoover Dam to Lake Mathews (MWD);
10. Understand N and P in downstream reservoirs (MWD);
11. Anticipate P budgets in water shortage scenarios;

12. Monitor zooplankton, phytoplankton, attached macrophytes, and algal toxins;
13. Conduct algal productivity assays to evaluate responses to changes (at times of change).

3. **Recreation in Boulder Basin: Specifics include (1) water aesthetics, (2) human contact, (3) shoreline smell from quagga mussels, (4) fish productivity, and (5) public perception.**

Evaluation actions are:

1. Conduct Lake Mead NRA visitor surveys;
2. Monitor quagga mussel presence and effect on recreation;
3. Monitor odors and identify causes;
4. Monitor water surface elevation and effect on recreation;
5. Monitor pathogens at beaches and in effluent (ongoing NPS monitoring) and in SNWA intakes;
6. Develop index of algae and recreation on swimming, boating, fishing (Toxic algae index already developed by SNWA);
7. Develop indices related to fish productivity;
8. Storm water monitoring;
9. Basic recreation use data;
10. Public perception.

4. **Ecosystem health in Las Vegas Wash, Las Vegas Bay, and Boulder Basin: Specifics are to (1) maintain ecosystem health and recreation quality and (2) fishery, wildlife, and vegetation in Las Vegas Bay and Boulder Basin.**

Evaluation actions are:

1. Monitor flow in Las Vegas Wash Tributaries and LV Bay (ongoing by USGS and SNWA);
2. Wash and Bay quality for NPDES regulatory compliance (ongoing by CWC members);
3. Monitor other water quality constituents with impact on recreation, fish, and wildlife;
4. Monitor the individual effluent quality from each plant (ongoing by each CWC member);
5. Understand the appropriate types of fish and wildlife to be supported;
6. Bio-monitor the fish, birds, and invertebrates (ongoing USFWS, SNWA);
7. Monitor zooplankton and phytoplankton;
8. Understand the flow and quality of urban runoff, shallow ground water, private discharges and stormwater in the Wash;
9. Monitor lake levels;
10. Model the changing conditions;

11. Understand Nevada Division of Environmental Protection programs.

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Appendix III Details of AWWARF Quagga Mussel Workshop

From April 3 to 4, 2008, the AWWARF hosted a workshop on quagga/zebra mussel control strategies. The facilitated workshop brought together experts in the field of zebra and quagga mussels. The workshop provided for an exchange of ideas and a current understanding of current science regarding mussels. The results of the workshop provided 14 recommendations for research in order to provide a better understanding of control of mussels in infrastructure through chemical inactivation and barriers and population management within the natural environment. There were 34 experts invited to the workshop whom where professionals in the field of mussel research and control. In addition, there were 140 attendees of this two-day workshop, which stimulated great interest in this emerging issue in the southwest. The consensus from the workshop was that the research efforts are required to provide control and management of mussels in the United States. A $20 million proposal was recommended for submittal to AWWARF. The following is a list of recommended research projects:

1. **Determination of Viability in Quagga Mussel Veligers and Assessments of Chemical Treatment Efficacy.**

   Existing methods for the determination of viability of quagga mussel veligers are not standardized and lack sufficient accuracy and precision to have sufficient confidence in results from different sources. Because of the non-standard approaches used to determine viability, there have been few attempts to set criteria for either oxidizing or non-oxidizing chemicals that are available for the eradication or control of quagga mussel veligers. The development of a standardized method would allow water officials to assess the effectiveness of control strategies and to determine cost effective approaches for their facilities.

2. **Hydraulic Effects on Veliger Mortality in Engineered Systems.**

   Quagga mussel veligers are found in water pumped from Lake Havasu by the Central Arizona Project through the Mark Wilmer Pumping Station. The plant pumps water in a single pumping stage with a single impeller pump for a total lift of 824 feet. No veligers have been observed to have settled between the top of the lift and the Bous Hill Pumping Station 25 miles to the east. It is unknown if the veligers are experiencing mortality or injury and if so the mechanism of damage is unknown. It has been hypothesized that shear forces, rapid pressure change, gas embolism, cavitations, or rapid velocity change encountered during pumping could impact veligers. The stage at which injury occurs is not known. The MWD pump lift plant is close to the Mark Wilmer Pumping Station but has a lift of only ~ 200 feet. Both pump stations takes veliger-rich water from Lake Havasu but the MWD plant is experiencing heavy mussel infestation in the pump discharge in the canal while Central Arizona Project is not. While the pumping process at the Mark Wilmer Pump Station is somewhat unique, there are sufficient instances of pump lifts of similar magnitude in the Western United States that the investigation of this apparent control mechanism should be of interest to other water utilities.

3. **Quagga Mussel Vulnerability Assessment and Response Management Tool Development.**

Water systems in the west transport water over long distances and from multiple sources using a variety of structures, processes, and conveyance systems. These systems are at risk and many are already experiencing quagga mussel infestations. Water systems need to respond to this emerging issue in a timely and effective manner and currently no concise guidance is available. There are numerous tools for monitoring and control and agencies need to consider which are most effective give their particular situation and risk tolerance. Municipalities, water supply agencies, and natural resource managers throughout the country/world have experience with dreissenid species under a wide range of environmental and operational conditions. There is great potential to learn from the successes and failures of these groups in their efforts to address prevention, treatment, and/or remediation. Summarizing these case studies in a central document would facilitate the dissemination of these results.

4. **Demonstrate Alternative, Non-Chemical, Control Technologies for Quagga Mussels for Deployment at Water Treatment Facilities.**

Alternative technologies such as small pore self-cleaning filtration and UV disinfection have been demonstrated as effective controls for dreissenid mussels. A method for non-chemical exclusion of veligers is needed to prevent them from entering water-treatment facilities. These technologies are not being widely used, primarily for three reasons: (1) perceived novelty of the technology; (2) lack of confidence in the product; and (3) higher initial cost of application. The advantage of these technologies is the ability to treat large volumes of water while maintaining a small footprint with minimal or no waster of water. These technologies do not negatively interfere with the quality of the final product (i.e., production of trihalomethanes in drinking water) and they do not involve hazardous materials. Further, these technologies do not generally require regulatory approval for installation. In the case of the small pore self-cleaning filter technology, additional benefit would be the removal of silt particles from the incoming water.

5. **Molluscicides and Biocides for control of Dreissenid mussels in Water Resource Projects.**

Various molluscicides and biocides have been used in attempts to control the spread of these invasive species, to reduce the impact of molluscan species on manmade structures, and to reduce and prevent the spread of diseases that require a molluscan intermediate host. The mode of action for these pesticides varies, as compounds as diverse as metal salts to complex organic compounds have been used successfully, while some require detoxification/inactivation by adsorption onto clay particles: others can be allowed to dissipate naturally. A comprehensive synopsis of available molluscicides and biocides is needed to aid resources managers attempting to address dreissenid mussel invasions. Recent success in identifying bacteria and bacterial toxins that destroy dreissenid mussels should be enhanced and applied to western waters.

6. **Coatings and Materials for Control of Dreissenid Mussel Attachment in Water Resource Projects.**

Various coating and materials have been used in attempts to control fouling of surfaces by these invasive species, to reduce the impact of molluscan species on fabricated structures. The mode of action for these coatings and materials varies but can generally be classified as either ablation/erosion or non-adhesion. Ablative coatings slowly scour from the applied surface limiting colonization while non-adhesion coatings prevent successful attachment. A comprehensive synopsis of available coatings and materials is needed to aid resource managers attempting to address dreissenid mussel attachment.

7. **Response of Quagga Mussel Veligers to Limnological Variables.**

Relatively little is known about the life history, ecological and environmental requirements of quagga mussels with regard to their success or failure at invading new systems or as these conditions influence population densities. Most of the information that has been developed in the United States is derived from the Great Lakes region where the genus was first introduced to the continent. Temperature regimes and other limnological conditions in this region of the country can differ significantly or are currently threatened with invasion. Among the primary environmental variables that need to be considered temperature, at both ends of the spectrum, needs to be addressed within the context of these western systems. Aquatic systems located in desert regions will have water temperatures that far exceed those of the Great Lakes, while the hypolimnions of some of the deep reservoirs and their associated tailwaters will have temperatures that are less variable than natural systems. Limnological variables (e.g. salinity/specific conductance, ionic composition, ecosystem productivity, retention time, depth, irradiance) need to be considered in the context of this recently invaded region.

8. **Application of Biological Agents to Control Quagga Mussels.**

Biological control of invasive species can be one of the most effective means of preventing or mitigation the impacts of these species if an effective candidate can be identified, an application procedure developed and if it can be demonstrated that the proposed control agent does not pose a separate threat to the native or desired flora and fauna. Aquatic ecosystem management has a mixed record in the use of biocontrol agents, too often the organisms selected fail to control the target species to extent desired or the control agent itself becomes a nuisance. These failures are most often a result of having too little background information prior to release. Attempts to control mollusks and other biological problems in aquaculture ponds has resulted in the release of several species of Asian carp (grass carp, silver carp, black carp, and bighead carp) into the Mississippi River Basin. When care is exercised in stocking, sterile grass carp can be effective at managing aquatic plant growth, but can also easily denude systems of all vegetation when overstocked. The sliver carp has been knows to injure boaters as it “leaps” into the air in response to boat traffic but has had little success in algal control. The black carp has been used successfully to control snails in aquaculture, reducing parasitic

infections, but it has also been implicated in damage to native mollusk communities. The introduced round goby may be an effective predator on zebra mussels in the Great Lakes, but the broader ecosystem impacts are yet to be quantified. Bacteria-based biological control of dreissenid mussels has been demonstrated using ubiquitous soil bacterium, *Pseudomonas fluorescens*. A toxin produced by this species, has been up to 90% effective at killing dreissenid mussels in controlled experiments with limited impact on other trophic levels and did not impact other mussel species.

9. **Applying Knowledge of System Ecology in Control Strategy.**

   Using an ecosystem approach to quagga mussel control could reduce the impact on existing ecological resources, simplify compliance and contribute to the resilience of the ecosystem overall. This approach takes advantage of existing ecosystem resources, encouraging self correction.

10. **Quantitative Tools for Management of Mussels in Colorado River System.**

    Models can provide important insights into physical, chemical, and biological processes occurring in lakes, rivers, reservoirs, and other conveyances. These models can be used to predict the outcomes of alternative management activities on water quality prior to action implementation. Most models developed to date have been able to make reasonable predictions about physical and chemical water quality parameters and mixed results with regard to changes in biological conditions. A limited number of models have been developed in northern states that attempt to quantify the water quality impacts of zebra mussels.

11. **Quantitative Evaluation of Quagga Mussel Outreach and Education Activities.**

    Extensive efforts have been undertaken in an attempt to communicate to the public the risks associated with quagga mussel invasion as well as the actions that can be taken to reduce the spread of this invasive organism. While these programs have been widely disseminated, it is unclear what impact they are having and which programs are more or less successful. In order to determine the success of these programs a quantitative evaluation must be undertaken using appropriate survey techniques.

12. **Shifts from Planktonic to Benthic Regimes in Response to Quagga Mussel Invasion.**

    The arrival of quagga mussels in western reservoir systems has the potential to significantly alter the food web. Quagga mussels at the sediment-water interface could consume food resources currently used by zooplankton in the water column. While some of the organic matter consumed by quagga mussels will be returned to the water column during reproduction, overall the introduction could result in significant reallocations of resources, resulting in major changes throughout the food web.

13. Early Detection Methodology and Rapid Assessment Protocols for Quagga Mussels.

Rapid responses and early detection of invasive species has been helpful in reducing the impact of these species and could be useful in preventing successful colonization of quagga mussels invading new areas. Early detection requires two components: analytical techniques for the rapid processing of samples and a proactive monitoring protocol to collect those samples. To facilitate early detection the analytical technique(s) must be refined and tested to the point that they require a reasonable skill level to perform with confidence and the protocol for assessing systems must not be so cumbersome to limit its use.

14. Impact of Quagga Mussel Invasion on the Quality of Domestic Water.

Lake Mead is the source of domestic water used by more than 22 million people. About 90% of the domestic water supply for southern Nevada comes from Boulder Basin of Lake Mead. Quagga mussels have heavily invaded the lake and the population density continues to escalate. Findings from other locations where both quagga and zebra mussels exist indicate the potential for the dense population to alter certain water quality parameters, especially in deeper portions of lakes and reservoirs. Quagga mussel waste (pseudofeces) has the potential to significantly affect water quality. It is essential to learn as much as possible about the potential changes to water quality from pseudofeces to develop and/or change treatment processes based on future conditions.

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Appendix IV Quagga Mussel Monitoring Questions

Interagency biologists have identified the following questions related to the infestation of quagga mussels within Lake Mead:

Quagga Mussels

Characteristics and Life History

1. Describe quagga mussel basic life history and life cycles in Lake Mead.
2. How long can veligers swim before settlement in Lakes Mead and Mohave?
3. How fast are quagga mussels growing, is there any significant difference among seasons and basins?
4. Can quagga mussels in Lake Mead grow to 38 mm, like those in the Great Lakes area?
5. What is the reproductive potential of quagga mussels and how many recruitments occur each year in Lakes Mead and Mohave?
6. What is the sex ratio of quagga mussels in Lake Mead?
7. Where does the food/nutrient threshold lie such that quagga mussel growth cannot occur (i.e., zero growth)?
8. What are the differences between quagga mussels living in the deep and shallow areas, morphologically, physiologically, and ecologically?
9. What is the natural mortality rate of quagga mussels in Lake Mead?

Distribution and Colonization

1. What is the distribution and relative abundance of quagga mussels within Lake Mead and Mohave (veligers and adults)?
2. What substrates do quagga mussels use? What substrate types do quagga mussels prefer: rock or silt?
3. What are the depth gradients of quagga mussel settlement? Do they prefer shallow or deep areas, or is settlement preference more complex?
4. What are quagga mussel temperature gradient tolerances in Lake Mead, should the previous record for the Great Lakes be corrected?
5. What are colonization rates on various natural substrates?
6. What are the colonization rates on water facilities, such as dams and docks?
7. Is there a population range that appears to be a stasis, or “normal” range?
8. What are the trends (monthly and annual) in numbers of quagga mussels?
9. How do quagga mussels cope with the high sedimentation in Lake Mead?
10. Is water flow significantly affecting veliger settlement?
11. What are densities at different inflow areas?
12. What are the densities at outflow area (below Hoover Dam)?

13. Can veligers swim to or travel to upstream with a boat to the tributaries, such as Muddy River, Virgin River, and Colorado River, even the lower Las Vegas Wash? Can they survive well in these tributaries if they can reach to these currently pristine waters?
14. What is the translocation rate for adult quagga mussels?
15. Are populations in Las Vegas Bay responding to changes in water delivery and volumes?
16. What are the characteristics of populations deep in the lake, compared to those at other depths, prior to changes in wastewater deliveries?

Ecological effects: fish and wildlife

1. What is the energetic budget of a quagga mussel?
2. How have quagga mussels influenced food web dynamics?
3. How will quagga mussels influence food web dynamics?
4. Do quagga mussels demonstrate selective feeding behavior in Lakes Mead and Mohave?
5. Can quagga mussels bioaccumulate inorganic contaminants such as Hg and Se?
6. Can quagga mussels bioaccumulate organic contaminants such as PCB (polychlorinated biphenyl) and PAH (polycyclic aromatic hydrocarbon)?
7. Do carp and other fish species consume adult and juvenile quagga mussels? Are veligers part of any fish diet?
8. Do waterfowl and other aquatic dependent bird species consume adult and juvenile quagga mussels?
9. What is the quagga mussel mortality rate resulting from predators in Lake Mead?
10. Can a quagga mussel bed destroy necessary habitat for razorback suckers in Lake Mead?
11. Can Lake Mead's hypolimnion become anoxic due to quagga mussels, directly and indirectly?

Water quality impacts related to drinking water and recreational experience

1. What is the oxygen consumption rate of quagga mussels?
2. How much ammonia nitrogen does a quagga mussel excrete in Lake Mead?
3. How much dissolved phosphorus does a quagga mussel excrete in Lake Mead?
4. What is quagga mussel filtration capability in Lake Mead?
5. Are quagga mussels responding to nutrients from wastewater?

Quagga mussel prevention, control, and infrastructure maintenance

1. How many available coating materials have been tested for quagga mussel prevention?
2. How many coating materials have been identified as appropriate candidates for industrial application?
3. Are there die-offs of quagga mussel adults noted and what are the impacts of die-offs?

4. Have treatments (chemical and mechanical) been effective?

Based on the experience gained from dreissenid mussels invasion into other ecosystems (e.g., Black/Caspian Sea, Great Lakes, Hudson River, Mississippi River, St. Lawrence River), the ecosystem's response to quagga mussels will be substantially significant, though some aspects may not respond immediately (such as zooplankton and fish) at the early stages of invasion. At the same time, Lake Mead is very unique, experiences from other systems may not pertain to this reservoir. The questions related to ecosystem responses to quagga mussels in Lake Mead are listed below.

1. Are their significant differences in components affecting water clarity before and after invasion among different basins, and is this change attributable to quagga mussels?
    a. Do mussels affect suspended inorganic particles?
    b. Is there any difference in chlorophyll *a* concentration before and after invasion?

2. How is water chemistry impacted by quagga mussels in Lake Mead?
    a. How will different nutrients (Nitrogen vs. Phosphorus) respond to quagga mussels?
    b. How different forms of nutrients (Dissolved vs. Particulate) respond to quagga mussels?
    c. Can Lake Mead's nutrient budget be changed due to the excretion of mussels?
    d. Can organic carbon in Lake Mead be changed due to the presence of quagga mussels?
    e. What is the oxygen budget in Lake Mead following the invasion of quagga mussels?

3. Will a shift occur in plankton communities?
    a. Which species/group of phytoplankton will benefit and which one will be negatively impacted by quaggas?
    b. How do zooplankton respond to quagga mussels in regard to direct predation by quagga mussels and/or the indirect impacts effected by the change in phytoplankton biomass?
    c. Are the responses to quagga mussels the same between microzooplankton and mesozooplantkon?

4. Will a shift occur in benthic communities?
    a. How will the benthic community respond to quagga mussels [i.e., will there be loss due to infestation by quagga mussels or benefit because quagga mussels provide more habitats (e.g. shell)]?
    b. How will periphyton in the benthic community respond to quagga mussels?
    c. Is Asian clam *Corbicula fluminea*, an earlier invasive bivalve in Lake Mead, suffering due to direct competition of food, resources, and habitat?

5. How do aquatic plants respond to quagga mussels?
    a. Is the presence of quagga mussels responsible for increased growth of macroalgae *Cladophora* in Lakes Mead and Mohave?

6. How do fish communities respond to quagga mussels?
    a. Will the habitat of razorback suckers be degraded, will carp production increase?
    b. How will threadfin shad and striped bass respond to quagga mussels, are they going to decline because of the food shortages?

7. Will the pathways of endocrine disrupting chemicals, such as PCBs and Mercury, in Lake Mead be changed, as mussels can accumulate these organic and inorganic materials from both suspended particles and dissolved phases?
8. What is the carrying capacity of Lake Mead for quagga mussels?
9. What is the overall response of the ecosystem to quagga mussels?

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Appendix V Detailed Protocols for Quagga Mussel Monitoring

Quagga mussel monitoring methodologies will be described in two parts: 1) monitoring methods on adults and juveniles, and 2) Monitoring on Veligers.

2. Methods on Sampling and Monitoring Adults and Juveniles

Consistent sampling of quagga mussels in the benthic community provides information on their settlement, growth rates, survival and mortality rates, age analysis, and their impacts on water treatment facilities and marinas, as well as their potential impacts on the local ecosystems. The population dynamics of quagga mussels in Lake Mead could be affected by substrate composition, substrate texture, substrate type, depth, currents, light, temperature, pH, food quantity and quality, ionic concentration, and the composition of the complete benthic community. Different substrate materials and textures will affect the abundance of quagga mussels (Mills et al. 1996, Bailey et al. 1999, Wilson et al. 2006). Usually the type of the substrates present is a key factor in determining where quagga mussels are likely to attach and grow.

1.1. Field Sampling

1.1.1. Sampling Sites The sites selected for long-term monitoring are likely going to be determined by the various organization concerned with quagga mussel infestation. For example, LMNRA may be more interested in the infestation of quagga mussels in marinas and boats, whereas SNWA has interests in monitoring the Alfred Merritt Smith Water Treatment Facility and River Mountains Water Treatment Facility because these facilities provide drinking water to millions of people living in Las Vegas area. One common interest to the lake resource managers, industries, public, and research scientists is the infestation status of quagga mussels in the whole Lake Mead. Knowing the abundance, distribution, growth, and recruitment of quagga mussels in general, we can anticipate how these invasive mussels are impacting on the Lake Mead's natural value (i.e., fisheries, benthic community, and planktonic community), cultural value (i.e., water quality), and recreational value (i.e., boat disinfection, unfavorable smells from mass mortality of quagga mussels). In order to get an overall estimation of the abundance and distribution of quagga mussels in Boulder Basin, Lake Mead, samples from transects in 7 locations (Figure 17) with different sediment types such as rocks, alluvial (unconsolidated sand and gravel), and muddy/silty sediment need to be collected.

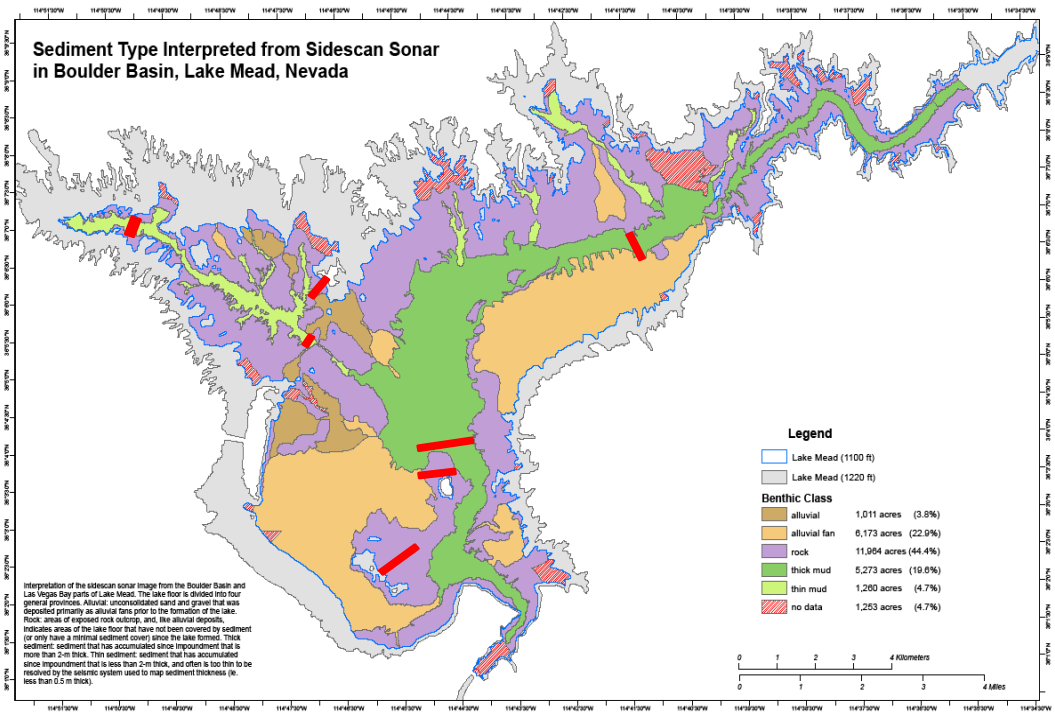

Figure 17. Sub-surface composition of Boulder Basin, Lake Mead (Twichell et al. 1999) and quagga mussel sampling locations (red bars represents the transects).

Mussels settle on hard substrate and tend to prefer dark areas, corners, crevices, and the shells of other mussels (Marsden 1992). Thus, hard substrates (i.e., rocks and stones) are expected to have more mussels than those with less compaction such as silt and mud. The composition of the sampling sites (i.e., rock, cobble, mud, silt, and sand) should therefore be recorded and used as references for setting up sites where the mussels will be collected. Based on USGS sediment type data in Lake Mead (the calculation for Boulder Basin is finished but the calculation of sub-surface coverage of other parts of Lake Mead is still in progress), the proportion of rock, sand and gravel (alluvial), mud, and others are 44.4%, 26.7%, 24.3%, and 4.7%, respectively. From these 7 locations, 16 samples will be collected in the rocky areas and 15 samples will be collected from the soft sediments (sandy and silty areas).

Lake Mead is the largest reservoir by volume in USA and is the second largest in terms of surface area (660 km^2) (LaBounty & Burns 2005). The sediment composition is heterogeneous in Lake Mead and its sub-surface is much larger than its surface area. Why 32 samples are sufficient to represent the real quagga mussel population in Boulder Basin? The more samples we take, the more representative the result is. However, economic and physical realities quickly set in. A standard simple random sampling design (Eaton et al. 2005), which is often advantageous to determine the number of samples necessary for a certain level of precision, has been be used to estimate how

many sampling sites (# of sampling sites) are enough to represent the population of quagga mussels in Lake Mead., which is often advantageous to determine the number of samples necessary for a certain level of precision, will be used to estimate how many sampling sites (# of sampling sites) are enough to represent the population of quagga mussels in Lake Mead:

$$\text{\# of sampling sites} = \left(\frac{T \times SD}{D \times Mean} \right)^2 \text{, where}$$

T = Tabulated T value at α level with the degrees of freedom of preliminary survey (generally α = 0.05)

SD = Standard deviation of preliminary samples

Mean = Mean density of preliminary samples

D = Required level of precision expressed as a decimal (0.30 to 0.35 usually yields a statistically reliable estimate)

For adults and juveniles, based on the National Park Service's preliminary data on quagga mussel sampling in the rocky, sandy, and muddy areas in 2007 (Bryan More, unpublished data), accepting a 5% probability of error (α = 0.05) and 137 degrees of freedom (138 samples were taken), the number of sampling sites needed to estimate quagga population is 56. This is satisfactory if the final estimates of mean quagga mussel density are correct within ± 35% (Table 6). That is, the results from 56 sampling sites will have a 95% confidence to estimate the real population density in Lake Mead. To have a higher confidence of 98% (α = 0.02) or 99 % (α = 0.01) on the final estimates of mean density, 79 and 97 sampling sites are needed (Table 6). α = 0.05 (98% confidence) will be used in the present quagga mussel monitoring plan in Lake Mead. Sampling from 56 sites is suggested and these sites should get the represent mean density of quagga mussels in Lake Mead (mean ± 35%, Table 6).

Table 6 Estimation of sampling sites in Lake Mead at different probabilities

A	T*	D* (Precision)	# of sampling sites needed
0.05	1.98	0.35	56
0.02	2.35	0.35	79
0.01	2.61	0.35	97

*T and D are described in the previous equation.

The sampling sites need to be proportionally representative of each sediment type. At least approximately 28 samples need to be collected from hard substrates (i.e., rocky areas) and another 28 samples from soft sediments (i.e., sandy, gravel, and muddy areas). Sites are selected in other basins of the lake by setting up transect lines perpendicular to the shoreline of Lake Mead and Lake Mohave (Table 7). In each transect, four to six samples from different depths will be obtained. Thus, in Lake Mead, the total sampling sites from different transects are 56 [=32 (Boulder Basin) + 6 × 4 (other basins)] and the first year samples are 152 [=32 × 4 (Boulder Basin) + 6 × 4 (other basins)]. This number gives a good representation of quagga mussels in Lake Mead.

Another factor that needs to be considered is the depth of each sampling site since depth could be an independent metric affecting the abundance and distribution of quagga mussels (Jones & Ricciardi 2005). Given the sampling effort and logistic input required, the priority of the present quagga mussel-monitoring plan will be primarily focused on Boulder Basin, as Boulder Basin is the most intensely monitored system by multiple agencies and the data generated can provide information for the BBAMP. Transects for collecting 32 samples from seven stations in Boulder Basin are suggested (Table 5). Monitoring transects in Virgin Basin, Overton Arm, Temple Basin, and Gregg Basin are also suggested. These sample sites are located at different depths covered with different substrates. The infestation impacts by quagga mussels can also be validated by the current water quality monitoring programs with minimal cost.

1.1.2. **Sampling Frequency** Monthly sampling is ideal to monitor the population dynamics of adult quagga mussels in Lake Mead. However, it may be too expensive to sample multiple sites so frequently. Based on the preliminary growth data (Wen Baldwin, unpublished data), most mussels in Lake Mead become sexually mature (> 10 mm) at least four months after settlement (although growth differs among different substrates). Therefore, it is suggested that sampling at a three-month interval, i.e., < 4 months, should be enough to monitor each mussel cohort in Lake Mead. According to the long-term temperature profile in Boulder Basin (LaBounty & Burns 2005), it is recommended that early February, early May, early August, and early November be used as the quarterly sampling times. After the first annual sampling, the data will be analyzed and can be used to determine if the sampling frequency should be decreased (e.g., every four months, every six months, or yearly sampling) or increased (e.g., bimonthly or monthly). For sampling locations in other basins (Table 5), the suggested sampling frequency is once per year in early November.

1.1.3. S**ampling Equipment** There are many kinds of quantitative sampling equipment: grab samplers, riffle/run samplers, core/cylindrical samplers, drift samplers, artificial samplers, and suctions samplers (Eaton et al. 2005). A quadrat frame is often used by divers to sample mussels *in situ* on water intakes, artificial samplers (e.g., concrete boards, tiles, and PVC pipes), rocks, and other hard

substrates. Each sampling approach has its own advantage and disadvantage when applied to different kinds of substrates. It is not appropriate for divers to collect samples in deep waters, while PONAR grab cannot work well on samples in bedrock or large cobble substrates. Based on the sub-surface cover sediment composition in Lake Mead, it is suggested that a combination of quadrat frame and PONAR grab are used in quagga mussel monitoring in Lake Mead. The equipment used for quagga mussel sampling includes:

1. GPS unit
2. Quadrat frames: small (0.1 m × 0.1 m, 0.01 m^2), medium (0.25 m × 0.25 m, 0.0625 m^2), and large (1 m × 1 m, 1 m^2)
3. PONAR Grab and a boat with a cable system
4. U.S. standard No. 30 sieve (0.595 mm opening)
5. Paint Scrapers
6. Zip-topped plastic bags (e.g., Ziploc®)
7. Markers to label bags
8. Heavy duty gloves
9. Large cooler with ice
10. Buckets
11. Mesh bags (mesh diameter < 500 μm)

1.1.4. Sampling Procedures Sampling procedures vary for each substrate and different densities of mussels (Marsden 1992). In Lake Mead, quadrats will be used by divers to collect quagga mussels in rocky areas, while PONAR Grab will be applied to areas containing sand and mud (Figure 17). The following methods are modified from those provided by Marsden (1992) for zebra mussel collection.

1.1.4.1. PONAR Grab Sampling in Muddy and Sandy Areas

Using the digital sampling map developed by UNLV and NPS, there are four stations with soft substrates (mud and sand). These 16 sites will be sampled using PONAR Grab techniques. For those sites in shallow areas (≤ 10 meters in depth), PONAR grab can be deployed by hand to dredge the sediments, while for those deep sites (> 10 meters in depth), the PONAR Grab will be deployed using a cable system from a boat. Specific procedures include:

A. Lower the open grab into the water until it touches the bottom, close the grab by releasing the messenger or trip line, then bring the grab to the surface. Place the U.S. standard No. 30 sieve underneath the grab as it leaves the surface to collect small organisms that may be lost as the water runs out. In the sandy sampling area, if the grab is brought to the surface partially open because of a big gravel (cobble) jamming in the opening, this sample should be discarded and another grab sample needs to be taken.

B. Place the full grab into the U.S. No. 30 sieve and rinse the grab with lake water until all the materials in the grab have been transferred into the sieve.

C. Position the sieve above a large bucket or other water containers that have been pre-filled with lake water. Wash the sediments using twisting and sloshing motion in the water. Keep all water below the rim of the sieve.

D. Place the cleaned materials into a pre-labeled zip-lock bag, refrigerate promptly on ice until transported to the laboratory for further analysis.

1.1.4.2. Quadrat Frame Sampling by Divers in Rocky Areas

In the digital sampling map, 16 samples from three transects in the hard substrates (rocky areas) have been identified. These sites with specific GPS locations will be sampled with quadrat frames by divers. In the case of sampling sites deeper than 120 ft, the samples will be collected with a remotely operated vehicle (ROV). Small, medium-sized, and large quadrats will be used for areas where the densities (individuals/m^2) of mussels are high (> 10,000), moderate (≤ 10,000 but ≥ 500), and low (< 500) (Table 7).

Table 7 Quadrat size and mussel density

Quadrat	Small	Medium	Large
Quadrat size	0.01 m^2	0.0625 m^2	1 m^2
Mussel density (Ind/m^2)	> 10,000	≤ 10,000 and ≥ 500	< 500
Number of mussels collected in the quadrat	> 100	≤ 625 and ≥ 31	< 500

Specific procedures include:

A. Place the quadrat frame on the surface based on the GPS specified location. Push the quadrat with force until the frame is firmly in contact with the substrate. If the substrates are vertical, place the quadrat frame against the surface.
B. Using a paint scraper, remove mussels inside the frame until none remain. These mussels could be individually attached to a rock or in a colony form. Caution should be exercised as some substrates may separate from the bedrock, also some mussels (e.g., tiny juvenile mussels) are not easily identified. If the diver is not certain whether there is a mussel on an underwater object, it is recommended that this object needs to be brought back for further examination in the laboratory.
C. Mussels are scraped into a mesh bag (mesh size < 500 μm) and transferred into a zip-topped bag (e.g., Ziploc®), promptly refrigerated and transported to the laboratory for further analysis.

1.2. Laboratory Measurement

After the collected mussels/sediments are transported to the laboratory, mussels need to be separated from each other carefully. Those mussels that are broken during collection and any dead ones (usually with open and empty shells) will be counted and discarded. Tiny juvenile mussels may attach on the empty shells. Accordingly, these dead mussels need to be examined carefully before being discarded. If possible, all mussels need to be counted and shell length recorded after they are transported to the laboratory. Following measurement, mussels need to be frozen at -20º C or lower for future biomass analysis. If limited by time or other factors, mussels collected from the field can be frozen until analyzed. There are two ways to quantify the density of quagga mussels: (1) number of individuals per square meter, and (2) biomass of mussels (dry weight or wet weight) per square meter. Although the first approach has been traditionally used, more and more evidence shows that the second approach has become more popular because the biomass method is more useful in evaluating the impacts of dreissenid mussels (Patterson et al. 2005, Burlakova & Karatayev 2008). Therefore, it is suggested that both the numbers and biomass of quagga mussels in Lake Mead should be recorded.

1.2.1. Density and Shell Length of Quagga Mussels

The sediment samples collected from each site in Lake Mead will be taken out of the plastic bag and thawed at room temperature (22º C) for 3 hours. Mussels that are visible will be picked and put on the top of a paper towel. All the sediments will be transferred into Petri-dishes and examined under a dissecting stereomicroscope to identify mussels that are not visible to the naked eye. The total numbers (**N**) collected from each site will be recorded using a click counter. Shell length of approximately 200 mussels (Marsden

1992) covering all the size ranges will be measured. The shell length of mussels > 4 mm is measured using a caliper while the shell length of those ≤ 4 mm is measured using a stereomicroscope with a micro lens fitted micrometer (Dermott et al. 1993).

1.2.2. Biomass of Quagga Mussels

It is unnecessary to measure the biomass of each individual if mussels are found in high densities. A random subset of 50 to 100 mussels can be used to represent different size categories at each site (Marsden 1992) and will be dissected for determining soft tissue and shell weights using preweighed weigh boats (Allen et al. 1999). If there is no tissue inside the shells, this mussel is considered as dead, and the data for the shell length (see section 1.2.1) will be discarded. Tissues and shells were dried to constant weight (2 days at 60º C in a drying oven) and weighed to 0.1 mg accuracy.

Small mussels can be combined to get a total weight and then divided by the number to get a mean weight. If so, the mean shell length of these small mussels also should be calculated. Based on the relationship of weight and shell length, equations will be developed (see section 1.3.3.) to estimate other samples. The weight of other unmeasured mussels will be calculated based on these equations.

1.2.3. Laboratory Equipment

1. Freezer (-20º C)
2. Stereomicroscope
3. Digital caliper
4. Weigh boats
5. Drying oven
6. Electronic balance
7. Paper towel
8. Click Counter
9. Forceps
10. Razor blades (Mounted in a handle)
11. Petri-dishes

1.3. Data Analysis

1.3.1. Density

The density of quagga mussels in the i^{th} sampling site will be calculated as:

D_i (Individual / M^2) = N_i / A_i, N_i is the number of mussels collected in the area, A_i is the square meter at the ^{i}th sampling site (it is 0.01, 0.0625, or 1 m^2 if small, medium, or large quadrat frame is used; it is the opening square of PONAR Grab if grab is used)

The mean density of mussels in each type of substrate and in the whole lake can further be estimated. The total numbers of quagga mussels in Lake Mead and a long-term trend can also be tracked.

1.3.2. Biomass

The biomass of a mussel is the sum of soft tissue and shell. The biomass of mussels (B_i) in each sampling site is the sum of the weighed mussels (see section 1.2.2.) and the un-weighed mussels generated from equations (see section 1.3.3.). The density of adult quagga mussels in terms of biomass in the i^{th} sampling site will be calculated as:

D_i (Milligram / M^2) = B_i / A_i, B_i is the mass of mussels collected in the area with A_i square meter at the ^{i}th sampling site (the same as section 1.3.1.). As above, a mean density in terms of biomass in each type of substrate and in the whole lake can be calculated. The total biomass of quagga mussels in Lake Mead and long-term trends can be obtained.

1.3.3. The Relationship between Shell Length and Dry Weight

Three regression equations can be constructed for each site.

1. Tissue weight and shell length.
2. Total weight and shell length.
3. Shell weight and shell length.

If the difference among different sites is not significant, or if the data is insufficient to generate a regression line (i.e., lower mussel density), data can be combined to form a line (i.e., for the same

substrate). Usually equations based on 1) on tissue weight and shell length and 2) total weight and shell length are more useful. These equations will be used to estimate the weight of those mussels that are not weighed (see section 1.2.2.).

1.3.4. Mortality, Recruitment, and Growth

Data on dead individuals, shell length, and biomass can provide key information on mortality rates, peak recruitment times/seasons in each year, and growth rates of quagga mussels at different seasons in Lake Mead. Many published references from the Great Lake region (Nalepa & Schloesser 1993) and Mississippi River watershed (Allen et al. 1999) can be used to estimate the recruitment and growth.

1.4. Ancillary Data Collection

The following limnological parameters of Lake Mead should be recorded while taking samples:

1. Water-level elevation (meters)
2. Specific conductance (µS/cm)
3. Secchi depth (meters)
4. Calcium concentration (mg/L)

At each sampling site, if possible, the following parameters should also be recorded:

1. Substrate type. If there is more than one type, try to describe as quantitative as possible with a percentage of each type. If there is no rock, the sediment grain size was classified into 3 major groups: sand (> 63 µm), silt (4-63 µm), and clay (< 4 µm) (Krumbein & Pettijohn 1938).
2. Sampling depth (meters)
3. Chlorophyll a (µg/L)
4. Dissolved oxygen in the water (mg/L)
5. Water temperature (º C)
6. Total phosphorus (TP: µg/L) and ortho phosphorus (PO_4-P: µg/L)
7. Total nitrogen (TN: mg/L) and nitrate (NO_3-N: mg/L)
8. pH
9. Phytoplankton community composition
10. Benthic macro-invertebrate assemblage

Most of these parameters (i.e., water-level elevation, specific conductance, Secchi depth, calcium concentration, chlorophyll *a*, dissolved oxygen, water temperature, total phosphorus (TP: µg/L), ortho phosphorus (PO_4-P: µg/L), total nitrogen, nitrate, and pH) are measured regularly by SNWA, CWC, and USBR as part of their regular water quality monitoring program. These parameters will be obtained on the date that is closest to the quarterly quagga mussel sampling date. SNWA's current phytoplankton community composition monitoring project should be sufficient to provide solid evidence to demonstrate the change of species (if there is any change) in Boulder Basin, although these stations are in different locations than the Quagga Stations. Samples of both substrate and benthic macro-invertebrate assemblage (living in both soft and hard substrates) will be taken back to the lab for further analysis.

2. Methods for Sampling and Monitoring Quagga Mussel Planktonic Veligers

The abundance of planktonic larval veligers can have a significant impact on the adult population (Schneider et al. 2003). The monitoring on veligers is as significant as monitoring on adults and juveniles. For early detection of quagga mussels, veliger monitoring is more important. The abundance and distribution of planktonic veligers are affected by many environmental factors such as temperature, food, current, and wave action (Claxton & Mackie 1998). Even minor changes in surrounding conditions can cause a substantial difference in the timing of production of ripe gametes and planktonic veligers (Nichols 1996). It has been documented that temperature is the key factor governing the gametogenesis and spawning of the dreissenid mussel. Larval production stops once water temperature drops below 10-12º C for zebra mussels (Nichols 1996), while quagga mussels can spawn at a temperature of 9-10º C (Claxton & Mackie 1998). In the Boulder Basin of Lake Mead, LaBounty and Burns (2005) have reported that the average water temperature in the epilimnion ranged from 12ºC in early February to 27º C in early August (the range is between 11 and 28.5ºC). The metalimnion average water temperature is between 12 and 18ºC and the temperatures within the hypolimnion are 12-12.5º C. Quagga mussel veligers are observed in Lake Mead in each month of the year, therefore, year-round veliger monitoring is recommended.

2.1. Field Sampling

Sampling Sites The distribution of planktonic veligers can be varied at different locations in Lake Mead due to environmental factors, such as food availability and flow hydrodynamics. Furthermore, in terms of volume (36.7×10^9 m^3, 100% capacity), Lake Mead is the largest reservoir in the USA (LaBounty & Burns 2005). The heterogeneity in water chemistry and hydrodynamics combined with the large volume of the lake means that a large number of sampling sites will better represent Lake Mead in its entirety than can a few sampling sites. . Preliminary USBR

data on average quagga mussel veliger densities in five locations in Lake Mead (Sandy Point, Echo Bay, Temple Bar, Hoover Dam/10M, and Hoover Dam/30M) from March to September 2007 and from January to June 2008 were used to estimate how many sampling sites are necessary (Chris Holdren and Denise Hosler, unpublished data) (Table 8).

Table 8 Estimation of veliger sampling sites in Lake Mead at different probability

α	T*	D* (Precision)	# of sampling sites needed
0.05	2.78	0.35	42
0.02	3.75	0.35	76
0.01	4.60	0.35	114

*T is the tabulated T value and D is the required precision expressed as a decimal

Based on Table 8, veliger sampling at 42 sites in Lake Mead will have a result (correct within ± 35%) at 95% confidence level. For a higher level of confidence, 98% (α = 0.02) or 99 % (α = 0.01), 76 and 114 sampling sites are needed respectively (Table 8). Currently, SNWA has 7 weekly veliger monitoring stations and USBR has five veliger-monitoring stations (Hoover Dam Shallow (0 - 10 m), Hoover Dam Deep (0 – 30 m), Sandy Point, Echo Bay, and Temple Bay) and an additional 19 sites with veliger counts (Table 3). The suggested weekly UNLV Sentinel Island station will provide more information on the veliger distribution at different depth of the lake. All these can provide a relatively complete picture on the early life history of quagga mussels in Lake Mead.

2.1.1. Sampling Frequency Veligers can be found any time of year due to warmer water temperature in Lake Mead. Weekly sampling to monitor veliger abundance at all stations is ideal because sampling at this frequency can track the peak density of veligers and won't underestimate the maximum veliger counts (Marsden 1992). However, given the cost of each sampling trip to multiple sampling sites, it is suggested that a monthly sampling frequency at each of the original five USBR sites be adopted in the present monitoring plan with the exception that weekly sampling take place at the NPS station near the USGS monitoring station.

2.1.2. Sampling Equipment Plankton nets are most commonly used to sample waters for veligers. The Wisconsin net (a removable bucket with a plunger that has to be removed to pour the contents of the bucket into a vial or bottle) and Student plankton net (having a funnel with a hose and clamp to empty the filtered contents of the net into a vial or bottle) are a few examples. A relatively large volume of water is reduced to a small volume, and the veligers will be concentrated. There are several protocols used in veliger collection, such as vertical plankton tow, and oblique plankton tow (Marsden 1992). An oblique plankton tow is used for presence/absence survey, or where the veliger

densities are extremely low. Pumped sampling is useful in shallow waters or large rivers where disturbed sediments or plankton blooms may clog a plankton net. Based on the current veliger status in Lake Mead, it is recommended that quagga mussel veligers be sampled with vertical plankton tow. The size of veligers in Lake Mead is greater than 75 µm (most of them are between 120 to 250 µm, David Wong, personal observation). Almost all the established zebra/quagga mussel veliger protocols call for a 63/64 µm net. Currently USBR is using a plankton net with 64 µm mesh size for veliger sampling. This size is consistent with what has been used by SNWA for zooplankton sampling in the past nine years. The equipment used for quagga mussel veliger sampling in Lake Mead includes:

1. GPS unit.
2. Plankton net (a Wisconsin net or Student net, with mesh size of 63 or 64 µm). Attach a wide-mouth Mason jar screw lid rim into the end of the net using a hose clamp. Alternatively, a mesh-lined plankton bucket can be used. Attach small lead weights to the hose clamp to ensure rapid sinking of the net.
3. Rope with length markers (e.g., 1m, 5m, 10m, 20m, 30m, 40m, 50m, and 60m).
4. Sample bottles (1000 mL Nalgene bottle is recommended).
5. Wash bottle (Squirt bottle).
6. Distilled or tap water.
7. 95% ethanol.
8. 1000 mL cylinder.
9. Sample labels.
10. Waterproof markers and labels.
11. Sieve made from a 250-mL plastic beaker with the bottom cut off and replaced with 64 µm plankton net mesh glued across the bottom.
12. 5-gallon bucket ("wash down" bucket).
13. 5-gallon bucket.
14. Big cooler (ice-chest) with blue ice.

2.1.3. **Sampling procedures** Using the digital sampling map developed by UNLV and NPS, these stations will be sampled with vertical and horizontal plankton tows. The specific procedures include:

A. For the five monthly monitoring stations, vertical plankton tow is conducted. Attach the rope to the "bridle" (the rope system fixed to the mouth of the net). Gently lower the plankton net to the water with a GPS-specified location (1m above the lake bottom). Retrieve the net at a rate of

approximately 1 m/second (a steady and unhurried hand-over-hand motion). Pulling too fast will cause a pressure wave in front of the net that pushes the water and plankton away from the mouth of the net, and as such, does not effectively sample the desired volume of water. Record the distance of each tow and use the diameter of the net and tow distance to calculate the volume of water filtered:

Water filtered (V_i: m^3) = $\pi \times r^2 \times H_i$

where π = 3.1416, r = radius of plankton net opening in meters, and H_i = distance through which net is pulled through the water in meters at the i^{th} GPS-specified location. For veliger sampling, a minimum of 1000 L of water needs to be towed; more volume is needed where veliger concentration is low.

For the weekly NPS monitoring station, all the samples at different depths (5m, 10m, 20m, 30m, 40m, 50m, 60m) are collected with vertical plankton net tow as described above. The surface water sample at this NPS station is collected with horizontal plankton net tow and 1000 L of surface water is suggested as the minimum volume. Therefore, in this station, the water volume collected will depend on the designated sampling depth.

B. Use lake water to wash down the outside of the net. When the net is clean, carefully remove the collection cup. Carefully unscrew the collection cup and pour sample into a pre-labeled (sampling date and GPS location) 1000 mL Nalgene bottle. Rinse the collection cup twice with Squirt bottle using minimal volume of distilled water, and put rinses into the same sample bottle.

C. Formalin is used to preserve the sample: If the total volume in the sample bottle is less than 500 ml, add distilled water to 500 mL and fill the bottle with 500 mL of 10% of formalin; if the total volume in the sample bottle is greater than 500 ml, use the 64 µm sieve to reduce to 500 mL and fill the bottle with 500 mL of 10% formalin. When ethanol is used to preserve the sample, if the total volume in the sample bottle is less than 750 mL, add distilled water to 750 mL and fill the bottle with 250 mL 95% ethanol; if the total volume in the bottle is greater than 750 mL, use the 64 µm sieve to reduce to 750 mL and fill the bottle with 250 mL of 95% ethanol. Either way, the final concentration of the preserved sample is approximately 5% formalin or 25% ethanol. Refrigerate the sampling bottle promptly in the cooler with blue ice until transported to the laboratory for enumeration.

D. To prevent cross-contamination and reduce the risk of spreading zebra and quagga mussels, all sampling gear, such as net, rope, and buckets that come into contact with the water, should be decontaminated. The gear should be soaked in 100% white vinegar (or 5% acetic acid solution) for one hour. Rinse with clean tap water (the bleach is corrosive so rinse thoroughly). Dispose of the contaminated rinse water away from the waterbody. The vinegar solution can be reused multiple times while the chlorine solution should be discarded after 24 hours.

2.2. Laboratory Counting

After the collected samples are transported to the laboratory, veligers will be quantitatively counted. There are several ways to count veligers. The 1000 mL samples can be counted by filtering through a sieve and backwash to a counting tray (Allen 1997), or subsamples can be taken for estimating the veliger density (http://www.usace.army.mil/). Listed below is a modified enumeration method currently used by USBR in Lake Mead (Holdren 2008b). It is a combination of Standard Method (10200 G) for the examination of water and wasterwater (Eaton et al. 2005), U.S. EPA Standard Method LG403 (USEPA 2007), and a method used by the U.S. Army Corps of Engineers (http://www.usace.army.mil/) (Chris Holdren and Denise Hosler, personal communication). Specific procedures are described below.

A. Mix the sample completely by swirling the 1000 mL sampling bottle. The sample is poured to an Imhoff settling cone with a venoset delivery system. Use distilled water to wash the sample bottle and add the rinses to the Imhoff cone as well. The sample is allowed to settle in the Imhoff cone for a minimum of 24 hours. If the sample contains a large amount of debris, it is recommended that the sample need to be filtered through a net as it is poured into the cone.

B. Successive aliquots of settled sample ($V_{concentrated}$: mL) are transferred into a centrifuge tube until no sediment remains in the Imhoff cone (usually the first 15 mL is enough, i.e., $V_{concentrated}$ = 15 mL).

C. Pipette a 1 mL aliquot from the well-mixed sample in the centrifuge tube using a Pasteur pipette (or Hensen-Stemple pipette). Dispense the aliquot into a Sedgwick-Rafter counting cell and carefully place the cover slip onto the counting cell perpendicular to the long axis of the slide. Slowly swing the cover glass so that it completely covers the sample well. Careful alignment of the cover glass will prevent air bubbles from being introduced into the sample and will ensure that the sample holds its complete volume (1 mL).

D. Place the filled Sedgwick-Rafter cell under a dissecting microscope with a click counter to record the number of veligers. A microscope with cross-polarized light is preferred because the veligers are identified more clearly using cross-polarized light. The arrangement of the calcite crystals, portions of the shell of

quagga mussel veligers in line with the axes of the cross polarizing filters do not reflect the light and thus the veligers appear with small glowing "Maltese" crosses (Figure 18). This requires a sub-stage light on the microscope. One polarizing filter is attached to the microscope objective lens, and another is placed between the substage light and the sample. Rotate the upper polarizing filter until the background is dark. Cross-polarized light will distinguish between *Dreissena* veligers and items found in plankton samples except *Corbicula* larvae, larvae of other dreissenids, and ostracods (refer to 2.3.). Examination of the counting cell is simplified if the cell is placed over a grid. A general examination of the contents of the cell will provide a feel for the density of organisms. If plankton densities are too high, it may be hard to see and identify any veligers. If densities are too low, time may be wasted in looking for veligers. Dilute or concentrate the concentrated sample as needed, taking care to record any dilution or concentration factors (X) (http://www.usace.army.mil/).

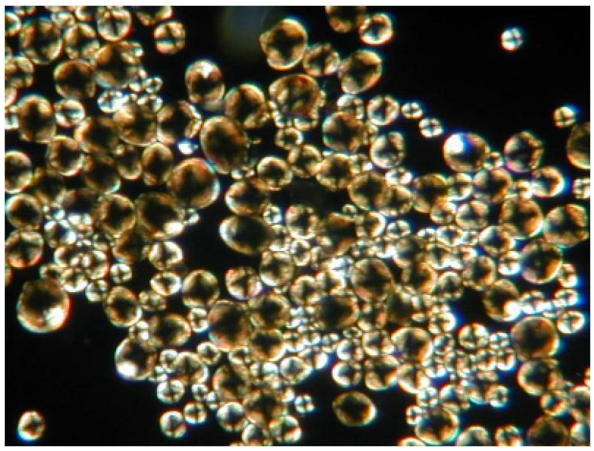

Figure 18. Cross-polarized light microscopy of quagga mussel veligers [Photo taken by Denise Hosler and presented in the interagency quagga mussel meeting (Holdren 2008b)].

E. This procedure for the same sample is repeated five times (5 replicates) and the mean value ($C_{concentrated}$: veliger/mL) is the veliger concentration of the 15 mL concentrated sample. Usually coefficient of variation (CV) among these five measurements less than 10% is acceptable (refer to 2.5.2.). X factor should also be recorded if further concentration or dilution is conducted.

2.3. Veliger Identification

In Lake Mead, there is only one *Dreissena* species, the quagga mussel *Dreissena bugensis*, ostracods are one of the major zooplankton groups (LaBounty & Burns 2005), and the Asian clam *Corbicula fluminea* is a major taxon in the benthic community (Peck et al. 1987). A microscope with cross-polarized light can distinguish between quagga mussel veligers and ostracods and *Corbicula* larvae. These organisms must be distinguished based on morphology, behavior, size, shape, or other features. Ostracods can be differentiated easily from

quagga mussel veligers by shape. Ostracods are more kidney bean shaped while veligers are either round or D-Shaped with a prominent straight hinge (Johnson 1995). In most cases, larvae of the Asian clam can be readily separated from those quagga mussel veligers based on presence of a foot, siphons, and shell size. D-shaped Asian clams are generally longer (240 μm) than D-shaped quagga veligers (108 μm). Unlike Asian clams, quagga mussel veligers never have a foot or siphons in combination with a straight-hinged shell. The difference in internal structures between these bivalve larval veligers can only be used to identify live, not preserved, animals (Nichols & Black 1994). Therefore, it is strongly recommended that DNA fingerprinting be used to distinguish the two groups of planktonic veligers in Lake Mead and further used to address veliger abundance and distribution at different time of the year.

2.4. Laboratory Equipment

A. Dissecting (stereo) microscope (one with cross-polarized light is preferred) with magnification to at least 40x

B. Sedgewick-Rafter counting cell (or plankton wheel)

C. Click Counter

D. Imhoff cones with a venoset delivery system

E. Centrifuge tubes (50 mL)

F. Disposable Pasteur pipettes (or Hensen-Stemple pipette)

G. Squirt bottle with distilled water

H. Dissecting probes

2.5. Data Analysis

2.5.1. Calculation of Veliger Concentration For the five USBR's monthly sampling stations, the total number of veliger in the i^{th} specific sample location is $N_i = C_{concentrated} \times V_{concentrated} \times X = 15 \times C_{concentrated} \times X$. X is the dilution or concentration factor. For example, if the 15 mL concentrated sample is diluted into 45 ml, then X = 3. If the 15 mL concentrated sample is further concentrated to 5 ml, then X = 1/3. The concentration of veligers in the i^{th} location (C_i: veliger/L) is:

$C_i = N_i \div V_i \times 1000 = 15{,}000 \times C_{concentrated} \times X \div (\pi \times r^2 \times H_i)$, where

C_i (veliger/L) is the veliger concentration in the i^{th} specific sampling location,

$C_{concentrated}$ (veliger/mL) is the mean veliger concentration of the 15 mL concentrated sample,

X is the dilution or concentration factor,

π is 3.1416,

r (meter) is the radius of plankton net opening in meters,

H_i (meter) is the distance through which net is pulled through the water in meters at the i^{th} GPS-specified location.

For the NPS station, the surface water is calculated the same as above with H_i as the horizontal tow distance. In order to get concentrations of veligers at different depth ($C_{0\text{-}5m}$, $C_{5\text{-}10m}$, $C_{10\text{-}20m}$, $C_{20\text{-}30m}$, $C_{30\text{-}40m}$, $C_{40\text{-}50m}$, and $C_{50\text{-}60m}$) for the NPS station, the total veligers collected from 5m (N_{5m}), 10m (N_{10m}), 20m (N_{20m}), 30m (N_{30m}), 40m (N_{40m}), 50m (N_{50m}), 60m (N_{60m}) will be firstly calculated with the formula $N_i = C_{concentrated} \times V_{concentrated} \times X$, where i = 5m, 10m, 20m, 30m 40m, 50m, and 60m, respectively. After that, the concentrations at different depth are calculated as:

$C_{0\text{-}5m}$ (veliger/L) = $N_{5m} \div V_{0\text{-}5m} = N_{5m} \div (\pi \times r^2 \times 5) \times 1000$

$C_{5\text{-}10m}$ (veliger/L) = $(N_{10m} - N_{5m}) \div V_{5\text{-}10m} = (N_{10m} - N_{5m}) \div (\pi \times r^2 \times 5) \times 1000$

$C_{10\text{-}20m}$ (veliger/L) = $(N_{20m} - N_{10m}) \div V_{10\text{-}20m} = (N_{20m} - N_{10m}) \div (\pi \times r^2 \times 10) \times 1000$

$C_{20\text{-}30m}$ (veliger/L) = $(N_{30m} - N_{20m}) \div V_{20\text{-}30m} = (N_{30m} - N_{20m}) \div (\pi \times r^2 \times 10) \times 1000$

$C_{30\text{-}40m}$ (veliger/L) = $(N_{40m} - N_{30m}) \div V_{30\text{-}40m} = (N_{40m} - N_{30m}) \div (\pi \times r^2 \times 10) \times 1000$

$C_{40\text{-}50m}$ (veliger/L) = $(N_{50m} - N_{40m}) \div V_{40\text{-}50m} = (N_{50m} - N_{40m}) \div (\pi \times r^2 \times 10) \times 1000$

$C_{50\text{-}60m}$ (veliger/L) = $(N_{60m} - N_{50m}) \div V_{50\text{-}60m} = (N_{60m} - N_{50m}) \div (\pi \times r^2 \times 10) \times 1000$

2.5.2. Calculation of Coefficient of Variation (CV) The coefficient of variation (CV) of the 5 measurements (1 mL each) is calculated as following:

$$CV\ (\%) = \frac{SD}{C_{concentrated}} \times 100, \text{ where}$$

$C_{concentrated}$ is the mean veliger concentration of the 15 mL concentrated sample,

SD is the standard deviation of the 5 subsamples and is calculated as:

$$SD = \sqrt{\frac{\sum_{j=1}^{5}(C_j - C_{concentrated})^2}{5-1}} = \frac{\sqrt{\sum_{j=1}^{5}(C_j - C_{concentrated})^2}}{2}, \text{ where}$$

C_j is concentration of the j^{th} subsample from 15 mL concentrated sample.

2.6. Ancillary Data

The following limnological parameters of Lake Mead should be recorded while taking samples:

1. Water-level elevation (meters)
2. Specific conductance (µS/cm)
3. Secchi depth (meters)

At each sampling site, if possible, the following parameters should also be recorded:

1. Chlorophyll a (µg/L)
2. Dissolved oxygen in the water (mg/L)
3. Current speed (meter/second)
4. Water temperature (º C) at different sampling depth
5. Total phosphorus (TP: µg/L) and ortho phosphorus (PO_4-P: µg/L)
6. Total nitrogen (TN: mg/L) and nitrate (NO_3-N: mg/L)
7. pH
8. Phytoplankton community composition
9. Zooplankton community composition

Most of these parameters (water-level elevation, specific conductance, Secchi depth, calcium concentration, Chlorophyll *a*, dissolved oxygen, water temperature, total phosphorus (TP: µg/L), ortho phosphorus (PO_4-P: µg/L), total nitrogen, nitrate, and pH) are measured regularly by SNWA, CWC, and USBR as their regular water quality monitoring program. For the five regular veliger-monitoring stations, these parameters will be obtained from USBR and SNWA. For the NPS station, most parameters can be obtained from USGS, SNWA, USBR, and CWC. SNWA's current plankton (phytoplankton and zooplankton) community composition monitoring project should be sufficient to provide solid evidence to demonstrate the change of species (if there is any change) in Boulder Basin.

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Appendix VI References

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